Winter 2011

Saprotrophic fungi as a mechanism for vertical nitrogen transport in a chronically fertilized northern hardwood forest

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SAPROTROPHIC FUNGI AS A MECHANISM FOR VERTICAL NITROGEN TRANSPORT IN A CHRONICALLY FERTILIZED NORTHERN HARDWOOD FOREST

BY

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Bachelor of Science, Environmental Science, University of New Hampshire, 2008

THESIS

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

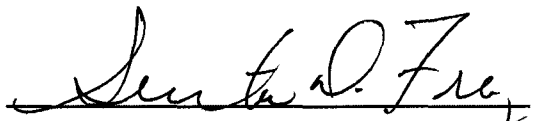
Master of Science

in

Natural Resources

December, 2011

This thesis has been examined and approved.



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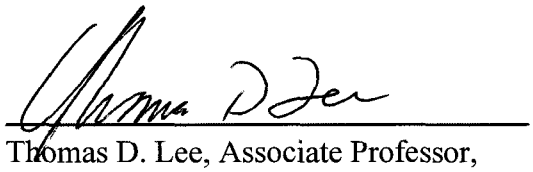

Thomas D. Lee, Associate Professor, Forest Ecology

20 Oct 2011

Date

DEDICATION

This thesis is dedicated to my parents, who have always supported my endeavors and have continuously encouraged me to live out my dreams.

ACKNOWLEDGEMENTS

I would like to thank my colleagues and friends—without them, this research would have never materialized. Special thanks to my lab mates: Mel Knorr, Sarah Andrews, Alix Contosta, Brian Godbois, Eric Morrison, Francesca Scandellari, and Lisa Greichen, for their support in lab and field; the folks in the Stable Isotope Laboratory, Andy Ouimet, Rachel Mixon, and Nikko Gagnon, for invaluable help with running the GC-IRMS; Matt Reuer at Colorado College for running ergosterol samples; my committee members, Tom Lee and Erik Hobbie, for providing research support and project ideas; and my best friends, Georgian Tutuianu and Ali Martin, for their emotional support and words of encouragement. Special thanks to Serita Frey, my long-term advisor, who has provided support, guidance, and encouragement throughout both my undergraduate and graduate degree programs.

Funding for this research was provided in part from The University of New Hampshire in the form of a two-year teaching assistantship and from the National Science Foundation.

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ABSTRACT

SAPROTROPHIC FUNGI AS A MECHANISM FOR VERTICAL NITROGEN TRANSPORT IN A CHRONICALLY FERTILIZED NORTHERN HARDWOOD FOREST

by

Katharine M. Burnham

University of New Hampshire, December 2011

Decay studies often observe that plant litter increases in the amount of nitrogen within the first year of decomposition, yet sources are uncertain. The Harvard Forest Chronic Nitrogen Amendment Experiment, Petersham, MA, was utilized to quantify vertical N translocation from soil up into decomposing litter and determine if long-term, chronic N application has affected this process. Litter bags were designed to facilitate or restrict fungal hyphal connectivity between the soil-litter interface and placed in control, low N+S and high N plots. After five or 12 months, a ^{15}N-$(NH_4)_2SO_4$ solution was horizontally injected into organic soil beneath bags. ^{15}N enrichment (i.e., translocation) of leaf litter was observed in high N fungi bags at 12 months. Similar quantities of fungal biomass-C across all N and litter bag treatments showed N translocation was not a factor of fungal establishment on litter, but rather leaf litter moisture, high soil-available N, and fungal hyphal bridges between the soil-litter interface.

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Introduction

Nitrogen is the most abundant element in Earth's atmosphere, hydrosphere, and biosphere. Nearly 99% is elemental N_2 and is unavailable to most organisms, except a group of N-fixing microbes (Galloway et al., 2003). Although usable N is limited, it is a compulsory component of biological molecules such as DNA, RNA, enzymes, and proteins; thus, most biological activities operate under N limitation. Thus organisms that evolved under N sparse conditions are physiologically adapted to survive and proliferate with limited N. Only recently have reactive (usable) forms of N—ammonia, ammonium, nitrate, nitric acid, NO_x, etc.,—become increasingly available, mostly a result of increased cultivation of legumes, combustion of fossil fuels, and the widespread use of the Haber-Bosch process for fertilizer and industrial activities (Galloway et al., 1995; Vitousek et al., 1997; Galloway et al., 2003). The combined effect of amplified abiotic and biotic nitrogen fixation have increased reactive nitrogen nearly 5 fold over the past 150 years (Galloway et al., 2003) and consequently altered N cycling through terrestrial ecosystems. In particular, the northeastern United States has received 3-10 kg N ha^2 yr^{-1} in wet + dry atmospheric deposition for at least the last several decades (NADP, 2011). The Harvard Forest Chronic Nitrogen Amendment Study (hereafter referred to as

"Chronic N") was established to study the effects of elevated N deposition on a forested ecosystem (Magill et al., 2004) and today serves as a unique opportunity to study how N limited processes have been altered. Fungal nitrogen translocation and litter decomposition are important N-limited processes, yet it is unclear whether increased N availability stimulates or depresses rates of either.

Saprotrophic fungi (decomposer fungi) form cord systems, also known as mycelia or hyphal networks, that develop in response to distance between resources, quality of resource, temperature, herbivory, and moisture (Boddy et al., 2009). Most importantly, saprotrophic fungi form extensive cord systems at the soil-litter interface in order to maximize resource utilization (Frey et al., 2003; Lindahl and Olsson, 2004; Boddy et al., 2009) and to overcome N limitations to the decomposition process. Movement of nutrients, such as nitrogen, from areas of greater resources (sources) to areas of lower resource availability (sinks) is a primary function of the mycelial network whereby the spatial heterogeneity of nutrients is reduced and the ecosystem becomes connected (Lindahl and Olsson, 2004; Boddy et al., 2009). Chronic and long-term application of reactive nitrogen may alter the substrate and decomposer community in unexpected ways such that source-sink dynamics and substrate quality drivers of decomposition may become altered.

Nutrient cycling and decomposition have been key areas of study at the Harvard Forest Chronic N Experiment. A litter decay study initiated in the fall of 1988 showed a 20-50% reduction in decomposition rates (k) of oak and maple leaf litter with increased N deposition and differences in mass loss became more apparent after 33 months (Magill and Aber, 1998). Cellulose and lignin mass of oak leaf litter declined throughout the 72

months, however, mass loss was suppressed in N addition plots compared to controls after only nine months of decay (Magill and Aber, 1998). In a similar yet shorter study, mass loss was found to decrease with increased initial litter C:N and lignin:N ratios where red maple and oak decomposed quickest, followed by pine needles and red maple wood (Micks et al., 2004). Nitrogen additions did not appear to significantly or statistically influence red maple or oak litter mass remaining at the end of the two-year period and 58-60% of maple litter and 43-50% oak litter mass remained (Micks et al., 2004). These findings (Magill and Aber, 1998; Micks et al., 2004) suggest that increasing N deposition does not dramatically affect the initial decomposition of higher quality litter (with more labile carbon compounds), but does suppress decomposition of low quality, recalcitrant, and highly decomposed leaf litter—especially after ~2 years of decay.

In conjunction with litter decay over time, Magill et al. (1998) observed black oak litter to increase in the absolute amount of N from 65 mg N to 100 mg N (~35% increase) within the first nine months for all treatments. With increasing time, however, control sites had greater mineralization rates than low N and much greater rates than high N litter (Magill and Aber 1998). Micks et al. (2004) observed no net change in N mass in decomposing oak leaves in either control or low N plots, despite initial immobilization in fertilized plots and initial mineralization in the control after two years. In N addition treatments, red maple litter increased in N but a decrease was observed in control treatments (Magill and Aber, 1998). Between 1990-1992, Micks et al. (2004) observed red maple litter N to increase ~25% within the low N plot, yet little change in controls. These studies demonstrate that N is initially immobilized into decaying leaf litter; however, the quantity of N immobilized is likely dependent on litter physiochemical

properties such as C:N and lignin:N ratios (Magill and Aber, 1998; Micks et al., 2004). Additionally, N fertilization appears to increase the quantity of N immobilized (Magill and Aber, 1998; Micks et al., 2004), yet both studies had no means of explaining the origin of exogenous N in litter (i.e., deposition, microbial N fixation, or fungal translocation).

Horizontal movement of nutrients by saprotrophs has been extensively documented (Wells and Boddy, 1995; Connolly and Jellison, 1997, Lindahl et al., 1999; Lindahl and Olsson, 2004; Lindahl et al., 2007; Hobbie and Horton 2007; Boddy et al., 2009); however, there have been few studies that have observed vertical movement of nutrients from soil up into decomposing litter (Hart et al., 1993; Frey et al., 2000; Frey et al., 2003). In a no-till agroecosystem field study, Frey et al. (2000) found that fungal translocation was an important inorganic N source and could account for net N immobilization observed in decomposing winter wheat straw. Similarly, Hart et al., (1993) observed a fungal translocation rate of 0.02 g N m^{-2} yr^{-1}, whereby inorganic N was moved from mineral soil up into the decomposing litter layer in a California mixed-conifer forest. These studies indicate that exogeneous N inputs during the initial stages of litter decomposition are likely the result of fungal translocation, but no studies have studied this mechanism or its importance in a temperate deciduous forest. This suggests the need for further research designed to test whether initial increases in exogeneous N during decomposition can be specifically and quantitatively attributed to fungal translocation of soil N in temperate forests. Hyphal connectivity between the soil-litter interface is, in most cases (i.e., Hart et al., 1993; Frey et al., 2000; Frey et al., 2003), assumed to be an important component of the system and likely the pathway for N import

into decomposing litter. However, there have been no studies that have mechanically prevented hyphal connectivity to determine the importance of this pathway.

Climate, litter chemistry, and soil organisms are main factors influencing decomposition (Aerts 1997). Decay rates in terrestrial ecosystems can be measured by soil respiration, litter fall/litter standing crop quotients (k_L-values), and direct weight loss measurements from litter bags (Aerts 1997). As such, several studies (Compton et al. 2004; Frey et al., 2004; Bowden et al., 2004) at the Harvard Forest hardwood Chronic N study have monitored decomposition and nutrient cycles and have observed biochemical changes of the soil community in N fertilized sites. Bowden et al., (2004) observed considerable increases of 26% and 24% in the amount of C respired from soil in low N (50 kg N ha^{-1} yr^{-1}) and high N (150 kg N ha^{-1} yr^{-1}) treatments, respectively, compared to the control (no N additions, ~8 kg N ha^{-1} yr^{-1} ambient atmospheric deposition) after one year of N fertilization. After 13 and 21 years of fertilization, soil respiration was depressed by 14.5% and 41% in low N and high N treatments (respectively), when compared to controls (Bowden et al., 2004; Scott Ollinger, personal communication). Decline in soil respiration over time with chronic applications of N was attributed to presumed declines in fine root biomass (Bowden et al., 2004). But in 2008, fine root (0-1 mm diameter) biomass in the high N organic horizon was greater (0.413 kg m^{-2}) than control treatments (0.142 kg m^{-2}; $p = 0.014$) and no other root size classes had significantly different root biomass between N treatments (Frey et al., in prep). These results indicate that reduced soil respiration cannot be attributed to a decline in root biomass and it is likely a result of decreased microbial biomass or a reduction of lignin-degrading enzyme activity (Frey et al., 2004).

Compton et al. (2004) found that nearly 10 years of N fertilization appeared to affect microbial biomass where fungal biomass, as determined by direct microscopy, in the A horizon was reduced from 6060 μg g^{-1} soil in control plots to 4742 μg g^{-1} soil in low N plots and to 3832 μg g^{-1} soil in high N plots; bacterial biomass increased from 174 μg g^{-1} in controls to 215 μg g^{-1} in the high N plots (Compton et al., 2004). The F:B ratio in the A horizon declined from 35.2 in controls to 24.1 and 17.5 with increasing N addition rates (Compton et al., 2004). After 15 years of N fertilization, there was no difference in total microbial biomass between N treatments or controls, while active fungal biomass was decreased from 7.5±0.9 μg CO_2-C g^{-1} soil in controls to 2.9±0.6 μg CO_2-C g^{-1} soil in high N treatments (Frey et al., 2004). The F:B ratio declined from 2.1 ± 0.4 to 1.5 ± 0.2 and 1.1 ± 1.2 μg CO_2-C g^{-1} soil in control, low N, and high N treatments, respectively (Frey et al., 2004). Moreover, high N fertilization decreased the utilization of N-containing substrates, such as L-amino acids and carbohydrates, yet increased utilization in low N fertilized plots (Compton et al., 2004). Depressed litter decomposition rates, decreases in CO_2-C respired from soil, suppressed active fungal biomass, and a reduction of lignin-degrading enzyme activity with increasing N additions are pieces of a larger story that suggests that increased N deposition greatly affects fungal activity at this site.

Although the litter bag method is over-simplified and does not provide a direct measurement of the decomposition rate, it is the most standardized method for studying the early stages of litter decay (Aerts, 1997). As noted previously, litter decomposition field studies usually observe an increase in inorganic N mass throughout the course of the experiment (Melillo et al., 1982; Magill and Aber, 1998; Micks et al., 2004), where

additional N may be accounted for by capillary flow, fresh leaf litter, fixation, absorption of atmospheric ammonia, throughfall, dust, insect frass, or fungal translocation and/or immobilization (Mellilo et al., 1982; Frey et al., 2000). Fungal translocation is thought to be a key contributor of exogenous N in the early stages of decomposition, as fungi must overcome N deficiencies for litter C utilization. Thus, the litter bag method not only serves as a mechanism for measuring litter mass lost over time, but could be designed to measure rates of fungal N translocation from the soil up into the decomposing litter.

Stable isotopes have been utilized to observe how nutrients cycle through ecosystems (Hart et al., 1993; Frey et al., 2000; Frey et al., 2003; Currie et al., 2004; Nadelhoffer et al., 2004; Hobbie and Ouimette, 2009). In the Harvard Forest, an isotopically enriched N solution was applied to control and low N treatments to determine if N fertilization affects long-term N and C cycling (Nadelhoffer et al., 2004). Recovered ^{15}N in live foliage 7 years after the labeling suggested that ^{15}N, and N in general, was continuously recycled within the system (Nadelhoffer et al., 2004). Moreover, as soils were the dominant sinks for all N inputs, N fertilization does not appear to stimulate increased C accumulation or act as a mechanism for increased C storage in woody biomass (Nadelhoffer et al., 2004). In a laboratory setting, Lindahl et al. (2001) used wood blocks labeled with ^{32}P and ^{33}P to observe simultaneous horizontal translocation of both P isotopes via a wood degrading fungi. Similar studies have utilized stable isotopes to quantify vertical movement of N (Hart et al., 1993; Frey et al., 2000; Frey et al., 2003) and C (Frey et al., 2003) in the field and lab. As such, isotopic enrichment of substrates is an ideal method for tracking total N translocated from the soil up into decomposing leaf litter through saprotrohpic fungi and may lead to a greater

understanding of how decomposing leaf litter initially increases in inorganic N. In this study, an isotopically enriched ^{15}N solution was applied to soil directly beneath litter bags in order to measure the rate of fungal N translocation from the soil up into decomposing litter after 5 and 12 mo. of decomposition.

Study Objectives and Hypotheses

The objectives of this study were to quantify vertical fungal N translocation from the soil into decomposing leaf litter and determine if long-term, chronic N deposition has affected this process. Secondary objectives were to measure decomposition and fungal biomass throughout a 12-month period. My hypotheses were

1. Increased N deposition suppresses decomposition rates, but not within the first year.
2. Increased N deposition increases soil N availability and decrease rates of fungal N translocation and decomposition.
3. Prevention of hyphal connectivity between the soil-litter interface reduces fungal N translocation from the soil up into decomposing plant litter.
4. Prevention of hyphal connectivity between the soil-litter interface impedes decomposition by increasing N limitation.
5. Increased N deposition negatively impacts fungal biomass, as the necessity to recycle N and the importance of decomposer fungi is reduced.

Methodology

Site Description

The Harvard Forest Chronic Nitrogen Amendment Study was established in 1988 to examine the long-term and chronic effects of elevated nitrogen deposition in an established temperate hardwood forest as part of a National Science Foundation (NSF) Long Term Ecological Research (LTER) site (Magill, Aber and Currie, et al. 2004). Experimental plots were delineated within a 50-year-old mixed hardwood stand located on the eastern side of Little Prospect Hill at the Harvard Forest in central Massachusetts (42°30'N, 72°10'W; Magill and Aber, 1998; Magill et al., 2004). Historical documentation of the area indicates that ~40-50% of the trees were damaged by the 1938 hurricane; post-hurricane trunk salvage was removed and slash remained unburned in the stand while the forest was allowed to regenerate naturally (Magill et al., 2004). Additionally, Harvard Forest timber records indicate that 3,005 boardfeet $acre^{-1}$ (17.75 m^3 ha^{-1}) of oaks were removed between 1942 and 1944 for firewood (Magill et al., 2004). Other tree species were likely harvested but saw-log volume is unavailable. The forest naturally regenerated to a mixed hardwood forest dominated by black and red oak (*Quercus velutina* Lam; *Q. rubra* L.) and lesser amounts of red maple (*Acer rubrum* L.), American beech (*Fagus grandifolia* Ehrh.), and black cherry (*Prunus serotina* Ehrh.). There is a lush understory of ferns, partridge berry (*Mitchella repens* L.), Canada mayflower (*Maianthemum canadense* Desf.), lowbush blueberry (*Vaccinium angustifolium* Aiton), maple leaf viburnum (*Viburnum acerifolium* L.), and striped maple (*Acer pensylvanicum* L.).

Dominant soils are stony-to sandy-loams, classified as coarse-loamy, mixed, frigid Typic Dystrochrepts of the Canton series (Currie et al., 1996; Magill et al., 2004). The landscape can be described as rolling with areas of exposed bedrock and shallow, acidic soils rich in organic matter.

Temperatures range from -12°C in January to 19°C in July with an average annual precipitation of 112 cm that is evenly distributed throughout the year (climate data available at http://harvardforest.fas.harvard.edu/; Magill et al., 2004). Daily temperature and precipitation data was obtained from the Fisher Meteorological Station at Harvard Forest (Boose 2001). Annual nitrogen deposition is 6.6 kg ha^{-1} yr^{-1} (2.2 kg ha^{-1} yr^{-1} as dry deposition) and has ranged from 6-8 kg N ha^{-1} yr^{-1} throughout the last two decades (NADP, 2011).

Four 30 × 30 m^2 plots were established in 1988 and each was subdivided into thirty-six 5 × 5 m^2 subplots. All subplots within a single plot received the same nitrogen treatment. The outer 20 subplots serve as a buffer and have not been included in most analyses, while the inner 16 subplots have been actively sampled. Since 1988, plots have been annually fertilized once a month for six months throughout the growing season (May – October) with NH_4NO_3 to increase N deposition rates to about 8 and 23 times greater than ambient levels. In this study, the lowest application of N (here after referred to as "low N") was 50 kg N ha^{-1} yr^{-1}, while the low nitrogen plus sulfur treatment (low N+S) received 74 kg S ha^{-1} yr^{-1} for the first 11 years and 50 kg N ha^{-1} yr^{-1} for all 23 years. The highest nitrogen treatment (high N) received 150 kg N ha^{-1} yr^{-1}, rates initially designed to stimulate N saturation. Control treatments received no N additions aside from ambient atmospheric deposition of about 6.6 kg N ha^{-1} yr^{-1} (Magill et al., 2004; NADP,

2011). Monitoring the effects of simultaneous nitrogen and sulfur deposition was a primary objective of the original N saturation study; however, 10 year baseline data indicated no difference between the low N and low N+S sites (Magill et al., 2004). Sulfur additions ceased in 1998 and the low N+S plot became a replicate low N treatment (Magill et al., 2004).

In 1989 and 1990, the control and low N plots were amended with ^{15}N to monitor long-term N storage and movement between soil and biota (Currie et al., 2004; Nadelhoffer, et al. 2004). The 30 × 30 m plots were divided in half and one side received labeled $^{15}NH_4^+$ and the other half received $^{15}NO_3^-$. Enough ^{15}N label was added such that $\delta^{15}N$ values were increased from 0 to 956‰ (ammonium, from 0.3663 to 0.7173 at. %) and 761‰ (nitrate, from 0.3663 to 0.6433 at. %). As the label was still detectable in the soil at the start of this study, the control and the low N plots were not utilized in an effort to avoid possible confounding factors such as externally labeled N sources. Off-plot controls were established nearby in the same forest community type; low N+S and high N plots was also used. Within the low N+S and high N stands, litter bags were installed in four random, inner subplots but did not interfere with permanent equipment (lysimiters, mini-rhyzatrons, and respiration collars). In total, 144 litter bags were installed into the plots and each replicate contained 12 bags, where six were fungi and six were no fungi bags. In this study, 89 bags were sampled: 26 from controls, 32 from low N+S, and 31 from high N plots. Uneven sampling numbers was a result of lost bags during the 12 month sampling.

Litter Bag Design and Installation

Two different litter bag types were utilized to either facilitate or inhibit fungal hyphal connections (hereafter referred to as "fungi" bags and "no fungi" bags, respectively) between the soil surface and decomposing leaf litter within bags. The use of a fungicide was decided against, as the scope of the project aimed at inhibiting hyphal connectivity between the soil-litter interface, while still allowing fungi to decompose litter. Fungi bags were constructed from white, no-see-um nylon tent mesh with 0.3 mm pore size diameter, while no fungi bags had a bottom constructed from white nylon mesh with 1 μm pore size diameter (Small Parts) and a top made from the same no-see-um mesh as the fungi bags. All bags measured approximately 12 × 12 cm^2 when stuffed with dry leaf litter and were sewn with black polyester thread on a Sear's Kenmore sewing machine. The large pore-size mesh allowed air, moisture, and small fauna movement in and out of the bag, while the small pore-size mesh served as a barrier between the soil-litter interface and restricted hyphal connections from the soil directly beneath the bag.

Leaf litter was obtained from the Harvard Forest Tom Swamp Tract, Compartment I, Hurricane Pull-down Experiment Control & Manipulated Plots in Petersham, Ma. All litter was collected and air dried in December 2007 and stored in paper bags until retrieved in August 2009. Litter was sorted by tree genus, the majority of which were *Quercus*, *Betula*, and *Acer*, cut with scissors into small pieces (~2 × 2 cm^2), and weighed into litter bags, such that each bag contained a litter composition of 75% oak, 15% birch, and 10% maple and weighed 6 g. Litter bag species composition was similar to the forest tree composition, but was restricted to the three dominant genera and was limited by available leaf litter. On May 20, 2010, litter bags were installed into field

plots in fungi–no fungi bag pairs. Surface litter was gently pulled away from the organic horizon surface and litter bags were mounted to the residual organic layer; litter bags were then covered with the native litter.

Soil Enrichment

The rate of N translocation from soil to litter was determined at two different times during the decomposition cycle—thus there were two incubation periods. Soil directly beneath litter bags was enriched with 98 at. % ^{15}N ammonium sulfate (Fisher Scientific) on October 28, 2010 or May 26, 2011 and enough label was added in an aqueous solution to enrich the soil inorganic ^{15}N pool to 10 at. % such that solution concentrations were 0.16 g m^{-2}, 0.43 g m^{-2}, and 0.97 g m^{-2} in the control, low N+S, and high N treatments, respectively. To ensure homogeneous solution application, a 12 × 12 cm^2 template with 25 evenly spaced points (Figure 2) was placed on top of each bag and used as a guide while solution was injected horizontally with a 22 gauge, 15 cm side port needle fastened to an air-tight glass syringe. The needle was inserted at a depth of 1 cm below the organic horizon surface and solution was injected in 0.1 ml increments at each point while pulling the syringe out of the soil. Seven days after the ^{15}N tracer was added, the litter bags and organic soil (12 × 12 × 2 cm^3 brownies) directly beneath litter bags were collected and brought back to the lab for analysis.

Soil Collection and Processing

The organic soil was passed through a 2 mm sieve to remove roots and rocks. Approximately 5 g of field moist, sieved organic material was dried in tins for 48 hours at 60°C and reweighed to determine gravimetric moisture (Equation 1).

Equation 1:

$$\text{Gravimetric moisture content } (g\ g^{-1}) = \frac{\text{Wet soil or litter (g)} - \text{Dry soil or litter (g)}}{\text{Dry soil or litter (g)}}$$

Within 48 hours, NH_4-N and NO_3-N were extracted from samples with 2 M potassium chloride and water and then gravimetrically passed through a #40 Whatman ashless filter. Filtrate was frozen and stored at -20°C until analysis. Ammonium was determined according to a modified indophenol-blue method described by Sims et al., (1995). Briefly, 175 µl of extracts were added in replicates of 8 to a clear 96 well microplate. Then, 25 µl citrate, 50 µl salicylate nitroprusside, and 25 µl hypochlorite were added consecutively to each well. Plates were covered and incubated in the dark at 25°C for 45 minutes and then analyzed colorimetrically at 650 nm absorbance using a BioTek Synergy HT microplate reader (Winooski, Vermont, USA). Nitrate was quantified by the vanadium (III) reduction reaction (Braman and Hendrix, 1989) modified for microplate assays (J. L. DeForest; according to Contosta et al., 2011). Briefly, 100 µl of extract was added in replicates of 8 to a clear 96 well microplate and mixed with 100 µl of vanadium (III) reagent. The plates were incubated in the dark at 25°C for 5 hours and analyzed colorimetrically at 540 nm. Soil pH (5 mo. samples only) was measured with a Corning pH Meter 430 by first mixing a 1:4 slurry of 5 g sieved

organic soil with 20 ml ultrapure deionized water, agitating the slurry for 15 minutes on a horizontal shaker table, and then inserting the pH electrode into the top portion of the slurry.

Litter Bag Sampling and Processing

Litter bags were gently wiped clean of outer debris, cut open along one edge, and inner contents were removed. Approximately 1-2 g field moist litter was immediately subsampled randomly, frozen at -20°C, and lyophilized for at least 6 hours. Lyophilized litter was ground to a fine powder with an 8000M Mixer/Mill (SPEX SamplePrep) for 5 minutes and used to determine ergosterol concentration, ^{13}C, ^{15}N, %C, and %N. Remaining litter was dried in plastic bags on the benchtop (~25°C) and assessed for moisture content (Equation 1). Total mass lost was determined on a dry mass basis after each destructive sampling.

Ergosterol concentration was determined according to a modified version of Bååth (2001). Approximately 200 mg ground litter was mixed with 2 ml MeOH and 0.5 ml 2 M NaOH, vortexted, and placed in a 70°C hotbath for 90 minutes. Then, 1 ml MeOH and 3 ml pentane were added to each sample, vortexed, and centrifuged. The clear, top liquid layer was decanted and placed in a separate, amber vial. The pentane-vortex-centrifuge-decant cycle was repeated twice more, adding only 2 ml of pentane each time. Amber vials containing decanted liquid were dried with N_2 gas and stored in a 4°C refrigerator until analyses could be completed. Samples were analyzed for ergosterol concentration at Colorado College. At Colorado College, samples were brought up to volume with HPLC grade MeOH (Fisher Scientific) and Milli-Q water, filtered with 0.2

μm syringe filters, and analyzed with a Waters Acquity UPLC with a C18 reverse phase column at 35°C (Matthew Reuer, personal communication). External ergosterol samples were prepared gravimetrically from solid ergosterol in methanol (Alfa Aesar). Ergosterol concentration was converted to fungal biomass-C by the conversion factor of 1 μg ergosterol g^{-1} litter is equal to 90 μg fungal biomass-C g^{-1} litter (Djajakirana et al., 1996).

Approximately 4 mg ground, homogenized litter was weighed on a Sartorius microbalance and tightly packaged into 8 × 5 mm tin cups and stored in a dessicator prior to analysis. Packaged samples were analyzed for ^{15}N, ^{13}C, %N, and %C on a Costech ECS Elemental Analyzer (EA) coupled to a Conflo III interface hooked up to a Thermo Finnigan DeltaPlus XP isotopic ratio mass spectrometer with a precision of 0.2 ‰ (University of New Hampshire Stable Isotopes Laboratory). Briefly, samples were sorted into an autosampler, individually dropped into a 1000°C combustion reactor whereby the sample (and tin cup) were completely combusted to CO_2 and then N gasses were reduced to N_2 in a reduction reactor at 650°C. Reduced gasses were carried via ultra high purity He carrier gas through a 3 m GC column, sorted within the Conflo III interface, and brought into the mass spectrometer for isotopic ratio measurement (SIL website, http://www.isotope.unh.edu/index.shtml). Internal standards were NIST 1515, NIST 1575a, Bolete, and Tuna and all were calibrated against N_2 reference gas. Stable isotope abundances are reported as: $\delta^{15}N$ or $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$, where R = $^{15}N/^{14}N$ of the standard (atmospheric N_2 for nitrogen and PDB for C) or sample.

The ^{15}N isotopic signature of nitrogen imported into litter bags (via translocation) was calculated by mass balance such that:

Equation 2: $\delta_f \times N_f = \delta_i \times N_i + \delta_a \times N_a$

Where δ is the ^{15}N value (‰), N is the mass of nitrogen, f stands for "final", "i" for "initial", and "a" for added (the immobilized nitrogen). The unknown mass of nitrogen added (translocated) was solved by:

Equation 3: $N_f = N_i + N_a$

Both natural abundance (unlabeled) and enriched (labeled) isotopic signatures of litter-imported nitrogen were derived.

Statistical Analyses

Two-way Analysis of Variance (ANOVA) was utilized to determine the effect of nitrogen treatment (control, low N+S, and high N) and litter bag type (no fungi and fungi) on gravimetric moisture content, C loss, N gain, $\delta^{15}N$, $\delta^{13}C$, active fungal biomass, and % leaf litter mass remaining. Natural abundance (unlableled) and enriched (^{15}N labeled) samples were analyzed together as the same treatment for all variables except $\delta^{15}N$; as such, n = 8 in all cases except for $\delta^{15}N$ when n = 4. Tukey's HSD post hoc analysis was utilized in all cases to determine significant differences between means.

Results

Temperature and Precipitation

Throughout the experimental year, temperatures ranged from a daily low on January 24, 2011 at -19°C to a high of 27.8°C on July 6, 2010 (Figure 3). For the 5 mo. sampling, the 7 day incubation period (defined as the time of soil labeling to the time of destructive soil and litter bag sampling; October 28 through November 4, 2010) had an average temperature of 5.2 ± 1.4°C and ranged from a high of 14.2°C on October 28th to a low of 1.6°C on November 3rd (Figure 4). During the 12 mo. sampling incubation period (May 26 through June 2, 2011), temperatures averaged 20.4 ± 1.0°C and ranged from a high of 22.6°C on May 27th to a low of 13.7°C on June 2, 2011 (Figure 4).

Precipitation (including snow liquid water equivalent) for the entire experimental period summed to 1.245 m and was evenly distributed thought the year (Figure 5). The fall incubation period began after 4 days of precipitation that amounted to 15.3 mm but there was no measurable precipitation during the incubation period except on the last day—while soil and litter bags were being collected—where 26.7 mm of precipitation fell as rain. The 12 mo. incubation period was initiated on a clear day after eleven consecutive days of rain (75.1 mm); 3 mm of rain fell in the middle of the week followed by 5 mm on the 6th day, but all other days were without precipitation.

Soil and Litter Moisture Content

Gravimetric moisture content was assessed for both organic soil and leaf litter within litter bags after 5 and 12 mo. of decomposition (Figure 6). Litter bag type (fungi or no fungi) did not affect soil moisture content directly beneath litter bags for either

sampling time. There was no N treatment (plot) affect observed during the 5 mo. (fall) sampling; however, at 12 mo. there was plot affect ($F = 45.4209$, $p < 0.0001$) where all treatments were statistically different ($p < 0.0001$) from one another. Controls had the lowest average moisture at 1 ± 0.04 g g^{-1}, while low N+S was 1.88 ± 0.02 g g^{-1}, and high N was 2.18 ± 0.13 g g^{-1}. Leaf litter moisture was statistically different ($F = 19.1383$, $p < 0.0001$) between litter bag types during the 5 mo. sampling, such that fungi bags were 0.4-1.3 units lower than no fungi bags. There was no interaction between N treatment plot and litter bag type as no fungi bags had greater litter moisture content (g g^{-1}) than fungi bags regardless of N treatment. In contrast, there was no difference in litter moisture content between bag types during the 12 mo. sampling.

A preliminary study (data not shown) in late August/early September 2010 supported assumptions that experimental sites were experiencing droughty conditions such that organic soil moisture in the control plots was of 0.6 ± 0.1 g g^{-1} while mineral soil (0-10 cm) was only 0.29 ± 0.08 g g^{-1}. Leaf litter in control bags was found to be even drier, where fungi bags had a moisture content of just 0.014 ± 0.008 g g^{-1} and no fungi bags were slightly greater at 0.021 ± 0.008 g g^{-1}. Visual observations revealed little to no hyphal ingrowth into litter bags during the preliminary study and no nitrogen translocation was observed in any treatment.

Soil Inorganic N and pH

Inorganic N ($NH_4^+ + NO_3^-$) in the organic horizon directly beneath litter bags (10/28/2010, 5/26/2011, and 6/2/2011) or next to litter bags (6/1/2010 and 5/7/2011) varied between date and N treatment (Table 1). Control soils ranged from a low of $3.99 \pm$

0.87 μg N g^{-1} soil in late October to a high of 18.38 ± 5.46 μg N g^{-1} soil in June 2010 and 18.07 ± 1.91 μg N g^{-1} soil in late May 2011. Low N+S soils ranged from 7.19 ± 1.35 μg N g^{-1} soil on October 28, 2010 to 41.7 ± 4.99 μg N g^{-1} soil on May 26, 2011. Low N+S soils always had more inorganic N than control but less than high N treatments. High N soils contained the greatest concentration (88.23 ± 11.92 μg N g^{-1} soil) in May 2011 and the lowest in June 2011 (29.48 ± 15.6 μg N g^{-1} soil).

Soil pH was measured for only 5 mo. samples (Table 2). There was no difference between N treatments or litter bag types; the average soil pH was 4.04 ± 0.00.

Leaf Litter Chemistry

Fresh leaf litter (mixture of oak, maple, and birch litter) had a starting C:N ratio of 55.76 ± 0.01, with 51.55 ± 0.45 % C and 0.92 ± 0.01 % N (Table 3). In total, each litter bag had approximately 3.09 g C and 0.06 g N at the start of the experiment. After 5 mo. of decomposition, the C:N was dramatically reduced and ranged from 39.99 ± 1.38 in no fungi bags in control treatments to 43.53 ± 1.43 in fungi bags in low N+S treatments—a 22-28% reduction (Table 4). Fungi and no fungi bags had a significantly different ($F = 9.9196$, $p = 0.0031$) C:N ratio at 5 mo. but there was no difference between N treatments. By 12 mo. of decomposition, the C:N was further reduced to between 28.68 ± 1.05 in no fungi bags in the high N treatment to 34.22 ± 1.73 in fungi bags in control treatments—a 39-49% overall reduction (Table 4). The C:N ratio was significantly different ($F = 4.7693$, $p = 0.0147$) between control and high N treatments; low N+S was not significantly different from either.

Isotopic data revealed that fresh litter had a $\delta^{15}N$ of -2.54 ± 0.04 ‰ and $\delta^{13}C$ of -29.47 ± 0.03 ‰. $\delta^{13}C$ changed little over the 12 mo. period and was only slightly enriched to an average of -28.94‰ (Table 4). Unlabeled litter throughout the experiment had average ^{15}N enrichment to -2.40 ± 0.08 ‰ at 5 mo. and -2.27 ± 0.11‰ at 12 mo. (Table 4).

Fungal Hyphal Growth and Nitrogen Translocation

Visual observations made during the 5 and 12 month samplings revealed that there was no hyphae growing from the soil up into no fungi bags and supports the use of 1 μm nylon mesh as an effective tool in preventing hyphal growth. In contrast, fungi bags with no-see-um nylon mesh allowed fungal growth from the soil up into litter bags; all fungi bags were observed to have hyphal connections between the soil-litter interface.

In all treatments, excluding control fungi bags, litter increased in N over the 12 mo. period (Figure7). In total, there was a 20-33% increase in total N from a starting amount of 0.055 g N to between 0.067 ± 0.002 g N in low N+S fungi bags to 0.073 ± 0.002 g N in high N fungi bags. Control fungi bags initially increased by 1.7% N but by 12 mo. had decreased 9.2% to only 0.05 g N per litter bag (Figure 7). High N fungi and no fungi bags had significantly more N than control fungi bags ($F = 2.5432$, $p = 0.0453$).

^{15}N enrichment of decomposing litter was observed in all treatments 1 week after soil labeling during the 5 mo. sampling (Figure 8). However, as no fungi bags were designed to restrict hyphal connectivity between the soil-litter interface, and thus prevent fungal translocation, it is unclear whether the enrichment observed in no fungi bags was a result of contamination, inadequate restriction of microscopic hyphae, or some other

enriched exogenous source. Because of this confounding factor, litter N enrichment cannot be specifically attributed to fungal translocation in these samples. During the 12 mo. sampling, there was no ^{15}N enrichment of litter observed in any treatment except in fungi bags in the high N treatment. $\delta\ ^{15}N$ was, on average, 1.6 ± 2.78 ‰ for the high N fungi bags, much greater that than the average -2.29 ± 0.12 ‰ of all other bags (Figure 8) but was not found to be statically different from unenriched, natural abundance litter in the high N plot or enriched litter from other N treatments ($F = 1.5380$, $p = 0.1714$). This insignificance is likely due to the small sample size ($n = 4$) and high variability, where one replicate had a $\delta^{15}N$ of 9.87 ‰ and the others three samples ranged from -2.13 to -0.58 ‰ and were not significantly enriched in ^{15}N. The high ^{15}N enrichment of one sample could not be explained by any factor or combination of factors, which suggests contamination. When the "contaminated" value was exempt from analyses, the average $\delta^{15}N$ was -0.30 ± 1.47 ‰ for high N fungi bags at 12 months and there was a strong difference between enriched and natural abundance high N litter $\delta^{15}N$ ($F = 20.739$, $p < 0.001$). Mass balances of litter-imported nitrogen revealed that there were no statistically significant differences in nitrogen ^{15}N values between natural abundance (unlabeled) and enriched (labeled) treatments for any combination of nitrogen treatment and litter bag type (Table 5).

Fungal Biomass-C

After 5 mo. of decomposition, fungal biomass-C averaged (across all treatments) 21.41 ± 0.87 mg g^{-1} leaf litter where biomass ranged from as low as 18.3 ± 2.3 mg g^{-1} in low N+S fungi bags to as much as 23.84 ± 2.68 mg g^{-1} in low N+S no fungi bags (Figure

9). Moreover, fungal biomass-C was observed to represent an average of 1.95 ± 0.04% of the total litter bag dry mass and ranged from 1.69 ± 0.31% in control fungi bags to 2.38 ± 0.27% in low N+S no fungi bags. After 12 mo. of decomposition, fungal biomass increased in all treatments; average biomass was 27.06 ± 0.62 mg g^{-1} leaf litter and ranged from 22.31 ± 5.71 mg g^{-1} in control fungi bags to 33.1 ± 2.83 mg g^{-1} in high N no fungi bags (Figure 9). Fungal biomass-C, as a % of total remaining leaf litter mass, averaged 2.8 ± 0.27% and ranged from 2.52 ± 0.2% in low N+S no fungi bags to 3.31 ± 0.28% in high N no fungi bags. There was no statistical difference between litter bag type or N treatment for fungal biomass-C (mg g^{-1} leaf litter) or as a % of total remaining leaf litter mass at 5 and 12 mo. Active fungal biomass was not correlative N translocation rates or % litter mass remaining. Additionally, soil or litter moisture content was not found to influence active fungal biomass at either sampling time.

Leaf Litter Decomposition

Percent leaf litter mass remaining was measured at approximately 5 and 12 mo. subsequent to the start date (Figure 10). While there were no differences in % mass lost between N treatments after 5 mo., no fungi bags had significantly less mass remaining and decayed at faster rates when compared to fungi bags ($F = 11.6931$, $p = 0.0015$). At 5 mo., fungi bags that received only ambient N had the most mass remaining (84.5 ± 1.1%), while no fungi bags that received 150 kg N ha^{-1} yr^{-1} had the least mass remaining (76.2 ± 1.51%). All other treatments had between 76.6-80.5% mass remaining and were statistically similar. There was no difference ($F = 0.4133$, $p = 0.6646$) in % mass lost (~69.7 %) between N treatments or litter bag treatments after 12 mo. of decomposition

(Figure 10). Overall, fungi bags that received only ambient atmospheric N decayed at a linear ($R^2 = 0.99$) rate of 4.56 ± 0.21 mg day^{-1} over the 12 mo. period; other treatments decayed at non-linear rates, where rapid, average decay rates between 7-8.5 mg day^{-1} were observed for the first 5 mo., yet slowed to between 0.9-4.6 mg day^{-1} from 5 to 12 mo. In general, neither elevated N deposition nor restriction of fungal hyphal connection between the soil and litter altered litter decay rates over a 12 mo. period.

Discussion

The main objectives of this study were to quantify the rate of fungal N translocation from the soil up into decomposing litter and to determine if long-term, chronic N application has affected this process. Secondary objectives were to simultaneously measure litter decay and fungal biomass, as these parameters were expected to affect fungal translocation. Litter decay rates observed within 1 year were not expected to vary between N treatments. However, fungal biomass was expected to decrease with increasing N deposition, whereby a decrease in fungal N translocation would be observed. Moreover, restriction of hyphal connectivity between the soil and litter interface was expected to suppress decay rates by limiting soil N availability.

Fungal N translocation was observed at 12 months in the high N fungi bags only. At this sampling point, the high N plot had greater available soil N and litter gravimetric moisture content (both fungi and no fungi bags) than all other treatments. Similar quantities of fungal biomass-C across all N and litter bag treatments shows that N translocation was not a factor of fungal invasion and establishment on litter. It appears that fungal N translocation occurs when the substrate is moist, soil available N is in excess, and fungal hyphae are able to form bridges between the soil-litter interface. Percent litter mass remaining after 12 months was similar across all N treatments, which implies that chronic long-term application of N fertilizer does not impede early stages of litter decomposition as expected. However, as no fungi bags had similar % litter mass remaining as fungi bags (regardless of N treatment), it appears that fungal connectivity between the soil-litter interface may not be as important for litter decay as previously

thought. Moreover, mass of N immobilized into decomposing litter was not correlated to % litter mass remaining, which implies that N did not limit decomposition in this study.

The year in which this experiment occurred was one of extreme climate. Average daily temperatures for summer and winter fell outside of the normal range for this area (Magill et al., 2004). Moreover, a droughty summer, snowy winter, and a wet spring further exacerbated soil conditions, prompting lower than expected microbial activity, likely due to stress. Total precipitation exceeded the annual average by 12 cm and, although it was evenly distributed throughout the year, summer precipitation events were stochastic and hot summer temperatures likely increased soil surface evaporation. A preliminary study (data not shown) carried out in late August/early September of 2010 revealed the O-horizon in control plots had an average moisture content of 0.6 ± 0.1, while mineral soil (0-10 cm) was only 0.29 ± 0.08, which support assumptions regarding drought conditions. Furthermore, the spring months were cold and soils were frequently inundated with water, as a precipitation event occurred 30 out of 56 days (54%) from April 1st through May 26th. Despite fluctuations in precipitation, both incubation periods began directly after a rain event with the intention of observing fungal activity. Soil and litter moisture at both sampling periods indicate favorable moisture conditions for fungal decomposition; however, prior conditions may have hindered fungal activity, N translocation, and decomposition rates.

At the end of the 12 months decomposition period, there was no difference in % mass remaining between any treatments. This is in agreement with Micks et al. (2004) who observed differences in % mass remaining between N treatments at this site only after about 2 years. Mass remaining were also similar to both Magill and Aber (1998) and

Micks et al., (2004), where about 70% oak litter remained in both ambient (control) and fertilized (low N) treatments, suggesting that long-term, chronic N deposition rates do not impede early stages decomposition. Samples collected at five months, however, revealed a rapid rate of decay for all treatments other than control fungi bags, suggesting that N application and restriction of fungal hyphae increases the rate of decomposition for a short period of time, as hypothesized by Aber et al., (1991). Magill and Aber (1998) observed N application to increase mass loss of oak and maple leaf litter within the first year of decomposition, but to retard rates after two years—which appears to be similar to the pattern observed in this study.

Interestingly, prevention of fungal hyphal connectivity between the soil and litter interface did not appear to impede decomposition rates as expected, but rather substantially increased litter decay rates within the first five months. In all cases, no fungi bags had less % mass remaining than fungi bags during the initial sampling. It is possible that no fungi bags facilitated a more favorable environment for decomposers such that slightly greater water was retained within the bags, keeping the litter substrate cool and moist throughout the droughty summer. This, in fact, held true during the preliminary study (control only) and for the five months sampling but not the 12 months sampling. As litter moisture content was correlated to % mass remaining during the five months sampling but not the 12 months—when there was no differences between treatments—quicker decay rates are likely a result of a moisture effect. It is also possible that the boundary between the soil and litter restricted soil fauna movement into and out of no fungi litter bags, restricting importation of foreign matter and fungal grazing. Similar

active fungal biomass across all treatments at both times, however, suggests that fungal grazing (if any) was consistent regardless of litter bag type.

Fungal biomass-C on leaf litter increased throughout the experiment. After 12 months, nearly 3% of the total leaf litter mass was fungal biomass-C in all treatments, indicating that chronic, long-term N deposition did not adversely affect active fungal biomass in this study. Similar fungal biomass-C concentrations have been observed in other studies; Scheu and Parkinson (1995) found to 35.4 mg fungal biomass-C g^{-1} dry aspen leaf residue in a Canadian boreal forest while Hieber and Gessner (2002) found up to 80 mg fungal biomass-C g^{-1} dry alder residue decaying in a freshwater stream. Fungal biomass-C of decaying leaf litter was expected to decrease with increasing N deposition as described by Frey et al. (2004) for soil; however, this was not the case for the litter sampled in this study. Similarities between N treatments may result from identical substrate quality (i.e., initial leaf litter quality was held constant for all treatments), insinuating that previous microbial biomass observations were a result of altered soil and leaf litter quality by site and not a direct effect of chronic N application. Fungal biomass-C of leaf litter was much greater than that observed for soil in previous studies (Compton et al., 2004; Frey et al., 2004), but this was to be expected, as litter is a microbial nutrient source.

Litter chemistry did not change throughout the year. Carbon content remained at about 50% and there was also little change in $\delta^{13}C$. However, total C mass did change significantly such that only about half C mass remained in control fungi bag treatments at the end of 12 months Percent litter mass remaining decreased linearly with greater C mass lost, which shows that litter decay is correlated with C degradation and/or

exportation. Contrary to what was hypothesized, there was no correlation between % litter mass remaining and g N gained or % N, which is contrary to previous findings (Magill and Aber, 1998). As such, it appears that N was not a limiting factor of decomposition in this study—at least within the 12 months timeframe—and is likely why there is no difference in % mass remaining between N treatments.

In natural, unlabeled samples, $\delta^{15}N$ did change over time, which indicates mixing of exogenous N with litter N. However, neither sampling dates—five or 12 months—indicated that imported N was derived from the soil with the exception of the high N fungi bags at the 12 months sampling. It is likely that in the high N treatment, greater soil moisture content in conjunction with availability of soil inorganic N both facilitated N translocation via fungi into decomposing litter. Frey et al. (2000) observed that fungal translocation, which was observed at much greater rates than here, could only account for a portion of imported N and suggested that atmospheric N deposition, inorganic fertilizer application, abiotic immobilization, or microbial N fixation on litter residues were potential contributors. If inorganic N fertilizer was a substantial N source, N treated litter (low N+S and high N) would have a greater N concentration than observed. While the control fungi bags did have a substantially lower % N and total N than other N treatments at the end of 12 months, control no fungi bags contained litter with the same % N and total N as low N+S and high N bags, suggesting that N fertilizer was not utilized and incorporated into decomposing litter or fungal biomass. Lower N mass in the control fungi bags at the end of 12 months is likely a due to greater rates of N exported into litter than imported, a process that was prevented in no fungi bags. Utilization of atmospheric N deposition and/or microbial N fixation on litter residues are likely occurring

simultaneously in the study and more research is necessary to assess and quantify these processes.

Fungal N translocation was minimal at most and only observed at the 12 months sampling in high N fungi bags. Greater availability of soil derived N and litter moisture content in the high N plot likely contributed to observed fungal translocation rates. Furthermore, similar masses of litter N in all treatments (excluding control fungi bags) at both five and 12 months suggests that N was not a limiting factor and decomposers did not utilize applied N as a source for decomposition. Active fungal biomass continued to increase with time but no differences between N treatment or litter bag type were observed. Litter decay rates were momentarily expedited within the first five months; however, this can be explained by increased leaf litter moisture content between N treatment and litter bag types. Control fungi bags followed a different pattern (compared to other treatments) for grams C lost and N gained. It is likely that the combined effect of litter moisture content and hyphal connectivity between the soil–litter interface facilitated these differences, yet they were not statistically significant. Overall, leaf litter decomposition nor the factors controlling decay rates were altered by chronic, long-term N application.

Alternative decomposition and fungal translocation rates would likely be observed if several aspects of this study were carried out differently. It is likely that decomposition rates and % mass remaining are different for each species of leaf litter utilized as observed by Magill et al. (2004) and Micks et al. (2004) for maple and oak leaves. There may be a stronger relationship between % mass remaining and N immobilized by litter species than that observed for the overall combined mixture of litter

species. However, design restrictions prevented adequate separation of litter species at either sampling for this relationship to be realized. Additionally, low N translocation rates observed may be a result of several factors including time and rapid abiotic N immobilization (Berntson and Aber, 1999; Dail et al., 2001). It is possible that the seven day incubation between soil enrichment and litter bag and soil collection was not enough time for sufficient utilization of the ^{15}N-$(NH_4)_2SO_4$. Some studies (Hart et al, 1993; Micks et al., 2004) observed $\delta^{15}N$ enrichment in decomposing leaf litter six months and two years, respectively, after soil enrichment while others (Frey et al, 2000; Frey et al., 2003) were able to see a strong fungal translocation signal after seven days. Moreover, previous studies from the Harvard Forest Chronic Nitrogen Enrichment Experiment revealed high rates of N immobilization, where 50% of the added ^{15}N-NO_3^- tracer was rapidly immobilized within 15 minutes in both control and high N treatments (Berntson and Aber, 1999). Micks et al., (2004) further reveals that within 15 minutes, 30-60% of an added $^{15}NO_3^-$ tracer disappeared from the extractible inorganic-N and solid phase organic-N pools of sterilized organic soils. Both studies strongly indicate alternative abiotic immobilization mechanisms are at work in Harvard Forest Chronic N soils. Although both studies used nitrate tracers, it is likely that an ammonium tracer, such as the one use in this study, would undergo similar abiotic immobilization in these soils—further compromising any observable signal.

Moreover, in situ experimentation is often times limited by external environmental factors, such as climate, that greatly affect the outcome of the study. Here, it is obvious that extreme variability in temperature and precipitation affected both sampling dates. However, field studies are extremely important in the scientific field, as

they are a keen reminder that observations revealed in laboratory experiments cannot be assumed mechanisms in nature.

Tables

Table 1. Inorganic N concentration (NH_4^+ and NO_3^-; μg N g^{-1} soil) in the O-horizon throughout the course of the experiment in control (0 kg N ha^{-1} yr^{-1}), low N+S (50 kg N ha^{-1} yr^{-1}), and high N (150 kg N ha^{-1} yr^{-1}) treatments at the Harvard Forest Chronic Nitrogen Amendment study. Values are means (n = 8) ± one standard error.

N Treatment	6/1/10	10/28/10	5/7/11	5/26/11	6/2/11
Control	18.38 ± 5.46	3.99 ± 0.87	15.05 ± 4.44	18.07 ± 1.91	NA
Low N+S	21.94 ± 3.44	7.19 ± 1.35	39.95 ± 12.48	41.7 ± 4.99	15.99 ± 6.91
High N	34.57 ± 6.45	46.98 ± 6.74	88.23 ± 11.92	62.32 ± 6.82	29.48 ± 15.6

Table 2. Soil pH in the O-horizon. All samples were from the 5 mo. sampling only. Values are means (n = 8) ± one standard error.

Plot	No Fungi	Fungi
Control	4.01 ± 0.07	3.90 ± 0.07
Low N+S	4.12 ± 0.09	4.08 ± 0.07
High N	4.04 ± 0.09	4.09 ± 0.08

Table.3. Initial leaf litter chemistry for litter bags comprised of oak, maple, and birch leaves. Values are means (n = 2) ± one standard error.

	$\delta^{15}N$	%N	$\delta^{13}C$	%C	C:N	Litter (g bag^{-1})	N (g bag^{-1})	C (g bag^{-1})
Fresh litter	-2.54 ± 0.04	0.92 ± 0.01	-29.47 ± 0.03	51.55 ± 0.45	55.76 ± 0.01	6.0 ± 0.01	0.055 ± 0	3.09 ± 0.03

Table 4. Leaf litter chemistry for the 5 mo. (A, top) and 12 mo. (B, bottom) samplings. Percent N, %C, 13C and C:N are means (n = 8) ± one standard error. $\delta^{15}N$ (no tracer, and tracer) values are means (n = 4) ± one standard error.

A. Plot	N (kg ha^{-1} yr^{-1})	Litter bag	$\delta^{15}N$, no tracer	$\delta^{15}N$, tracer	%N	^{13}C	%C	C:N
Control	0	No Fungi	-2.48 ± 0.05	-1.59 ± 0.85	1.27 ± 1.61	-28.91 ± 0.10	50.30 ± 0.19	39.99 ± 1.38
		Fungi	-2.50 ± 0.16	-0.88 ± 0.44	1.16 ± 0.03	-28.89 ± 0.09	50.22 ± 0.19	43.50 ± 1.22
Low N+S	50	No Fungi	-2.36 ± 0.11	-1.38 ± 0.87	1.25 ± 0.05	-29.04 ± 0.10	50.50 ± 0.19	40.72 ± 1.34
		Fungi	-2.47 ± 0.12	-1.90 ± 0.16	1.16 ± 0.04	-29.15 ± 0.29	50.22 ± 0.37	43.53 ± 1.43
High N	150	No Fungi	-2.25 ± 0.09	-1.50 ± 0.44	1.36 ± 0.04	-28.70 ± 0.11	50.23 ± 0.25	37.18 ± 1.24
		Fungi	-2.32 ± 0.08	-1.37 ± 0.16	1.24 ± 0.04	-29.21 ± 0.14	50.63 ± 0.17	41.15 ± 1.38

*only one replicate sampled

B. Plot	N (kg ha^{-1} yr^{-1})	Litter bag	$\delta^{15}N$, no tracer	$\delta^{15}N$, tracer	%N	^{13}C	%C	C:N
Control	0	No Fungi	-2.68 ± 0.01	-2.29 ± 0.05	1.61 ± 0.13	-28.89 ± 0.13	51.91 ± 0.12	33.05 ± 2.31
		Fungi	-2.53*	-2.67 ± 0.13	1.52 ±0.08	-28.81 ± 0.14	51.46 ± 0.59	34.22 ± 1.73
Low N+S	50	No Fungi	-2.14 ± 0.07	-2.16 ± 0.06	1.63 ± 0.05	-28.73 ± 0.09	51.42 ± 0.23	31.74 ± 1.11
		Fungi	-2.21 ± 0.14	-2.29 ± 0.11	1.59 ± 0.08	-28.97 ± 0.14	51.77 ± 0.21	33.16 ± 1.78
High N	150	No Fungi	-1.91 ± 0.11	-2.02 ± 0.08	1.82 ± 0.07	-29.32 ± 0.12	51.66 ± 0.15	28.68 ± 1.05
		Fungi	-2.19 ± 0.15	1.60 ± 2.78	1.79 ± 0.09	-28.94 ± 0.21	51.79 ± 0.23	29.34 ± 1.41

Table 5. δ ^{15}N values for litter-imported nitrogen at 5 and 12 months in control, low N+S, and high N nitrogen treatment plots with either no fungi or fungi litter bag type. Both time periods have natural abundance (unlabeled) and enriched (labeled) values for comparison. All values are means (n= 4) ± one standard error except for natural abundance control fungi bags at 12 months where only one sample was measured.

	Litter bag	5 Months		12 Months	
Plot	**type**	**Nat. Abund.**	**Enriched**	**Nat. Abund.**	**Enriched**
Control	No Fungi	1.40±3.75	23.09±23.83	-3.46±0.03	-1.30±0.258
Low N+S	No Fungi	3.27±1.70	8.18±6.59	-0.29±0.56	0.30±1.13
High N	No Fungi	1.24±0.73	8.48±2.06	2.10±1.56	-0.53±0.40
Control	Fungi	6.48±3.30	20.34±10.42	-2.42	-3.56±2.74
Low N+S	Fungi	7.60±2.57	16.77±9.93	-0.94±0.81	-0.94±0.69
High N	Fungi	4.27±4.60	27.49±23.62	-0.27±0.86	2.21±1.79

Figures

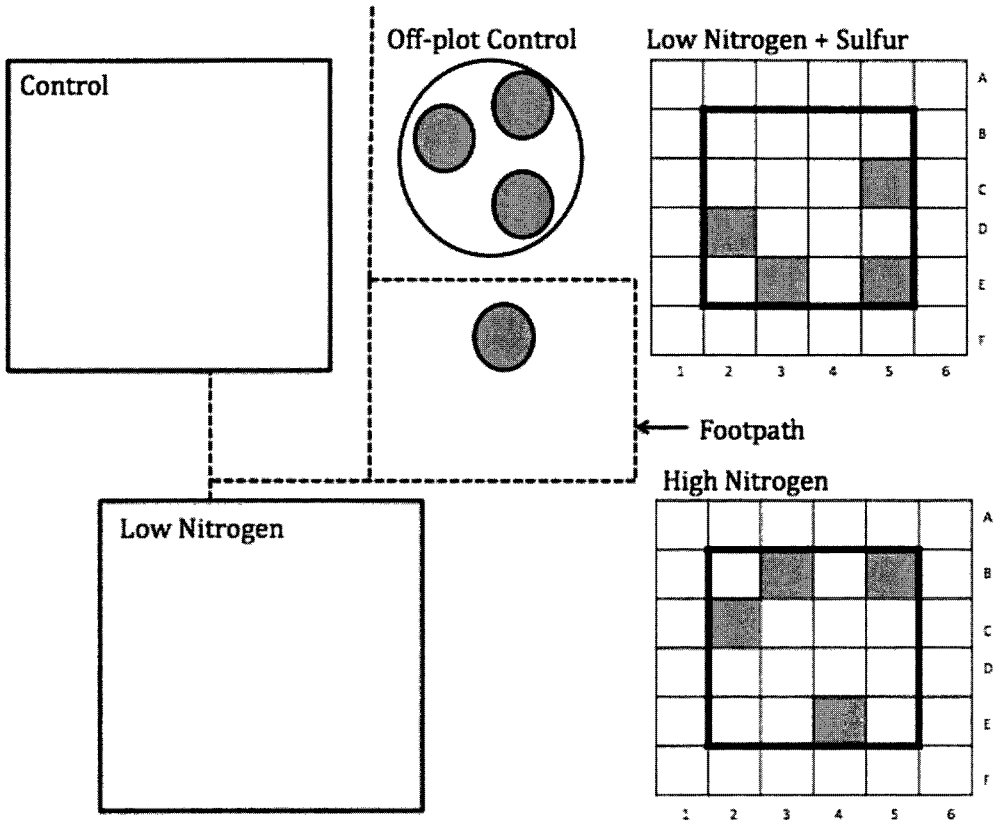


Figure 1. Harvard Forest Chronic Nitrogen Amendment Study experimental plot set-up. Four 30 × 30 m^2 plots were established in 1988 and sub-divided into thirty-six 5 × 5 m^2 subplots. In 2010, an off-plot control was established between the original control and low nitrogen + sulfur plots. Gray shaded areas represent subplots utilized in this study.

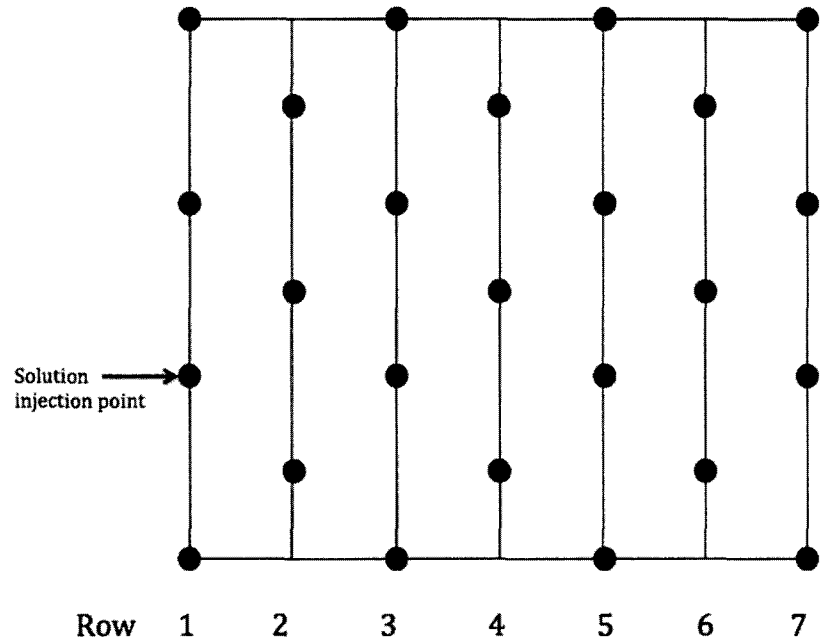


Figure 2. Solution injection template. 2.5 ml of ^{15}N labeled solution was heterogeneously injected into the soil at 25 points 1 cm below the organic horizon surface and directly beneath leaf litter bags.

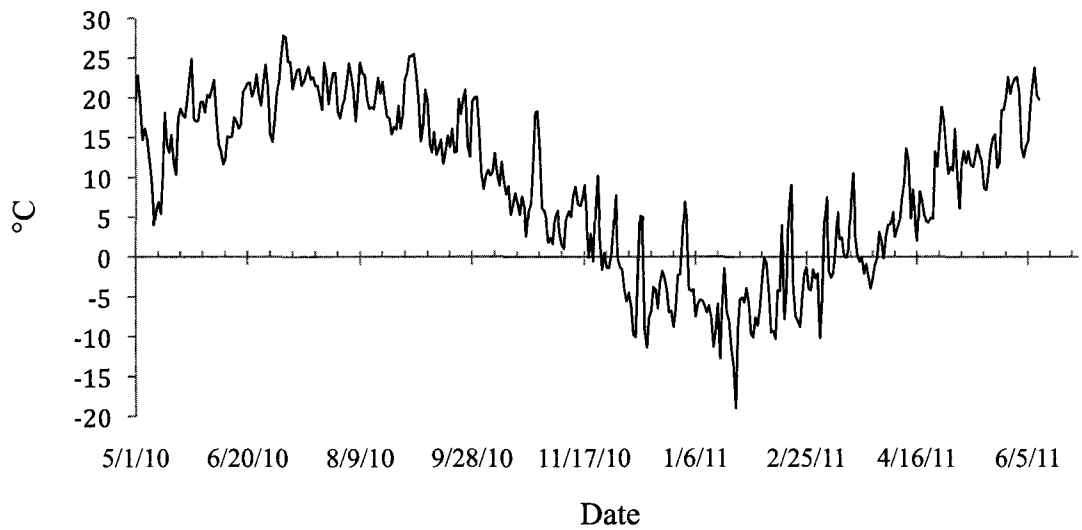


Figure 3. Daily average temperature from May 1, 2010 to June 10, 2011. Data taken from the Harvard Forest Fisher meteorological station, Petersham, MA, which is available on the web at http://harvardforest.fas.harvard.edu/hfmet/.

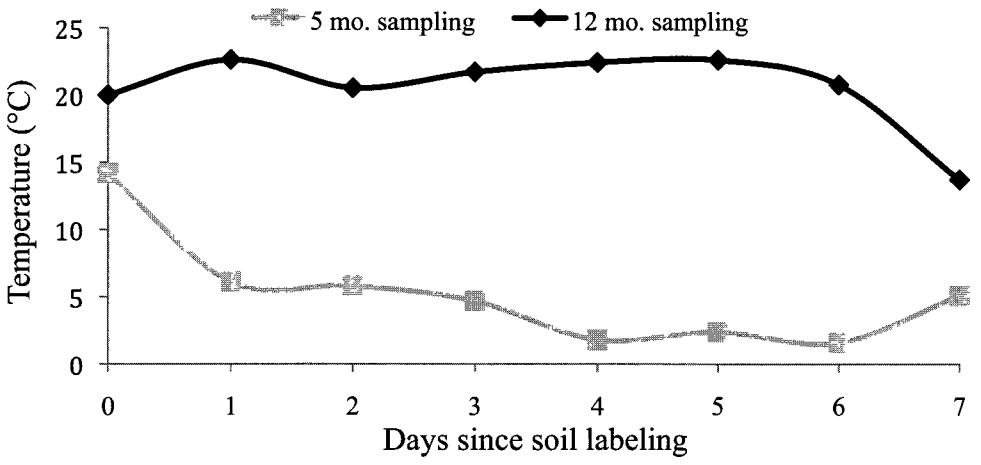


Figure 4. Daily temperature (°C) during the 5 and 12 mo. incubation periods.

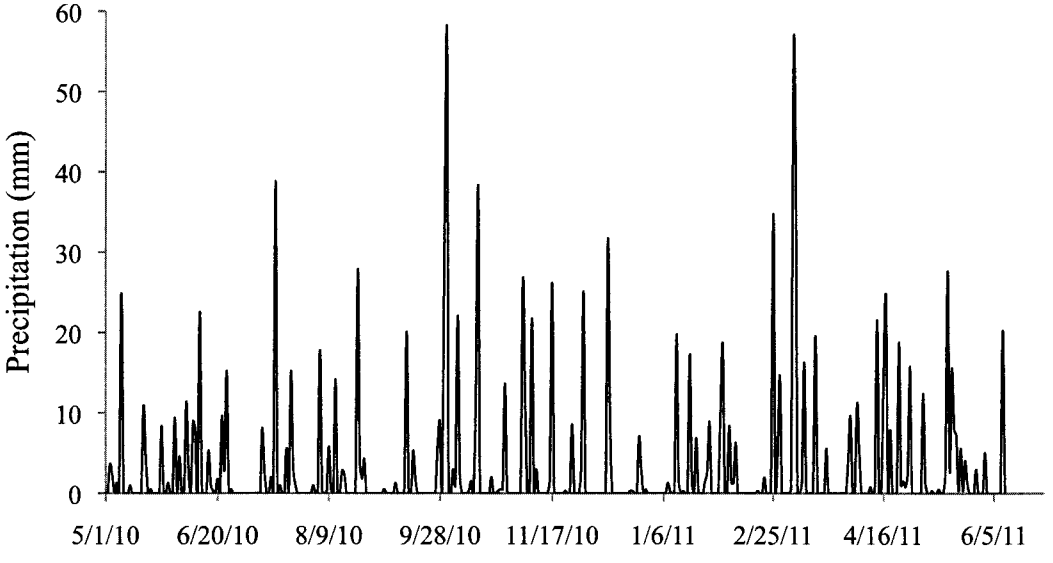
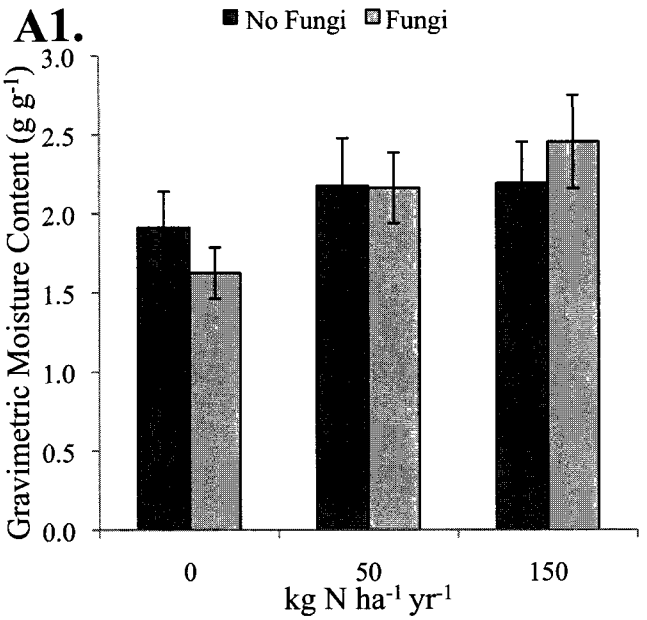
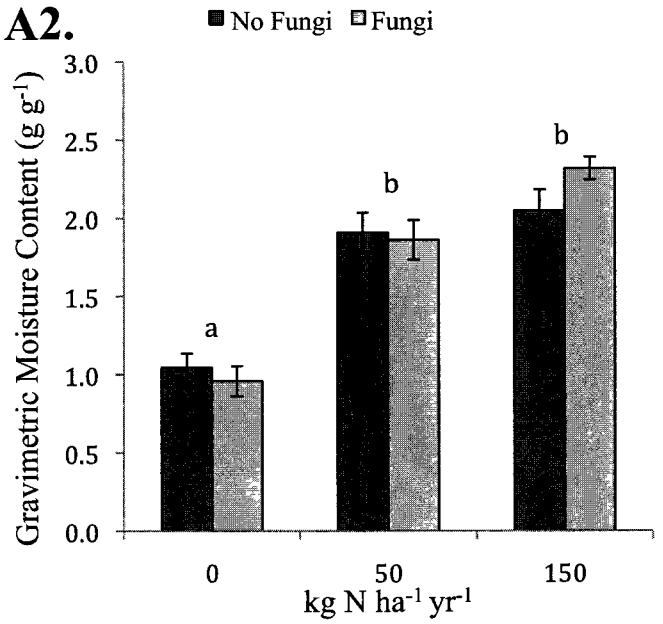


Figure 5. Annual precipitation (mm) from May 1, 2010 to June 10, 2011. Data taken from the Harvard Forest Fisher meteorological station, Petersham, MA, which is available on the web at http://harvardforest.fas.harvard.edu/hfmet/.


A1.
No Fungi
Fungi
Gravimetric Moisture Content (g g-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
150
kg N ha-1 yr-1


A2.
No Fungi
Fungi
Gravimetric Moisture Content (g g-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
b
0
50
150
kg N ha-1 yr-1

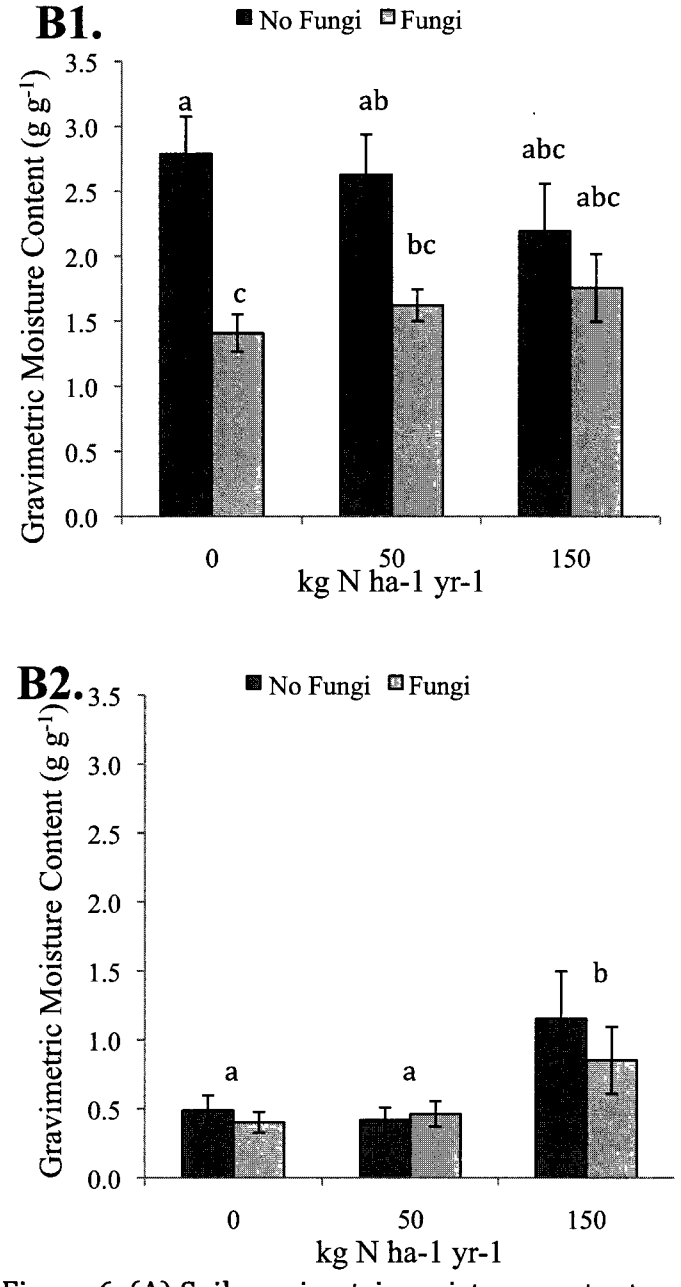


Figure 6. (A) Soil gravimetric moisture content and (B) leaf litter gravimetric moisture content for (1) 5 month sampling and (2) 12 mo. sampling in control (0 kg N ha^{-1} yr^{-1}), low N+S (50 kg N ha^{-1} yr^{-1}), and high N (150 kg N ha^{-1} yr^{-1}) treatments at the Harvard Forest Chronic Nitrogen Amendment study. Columns represent means (n = 8) and error bars are one standard error of the mean.

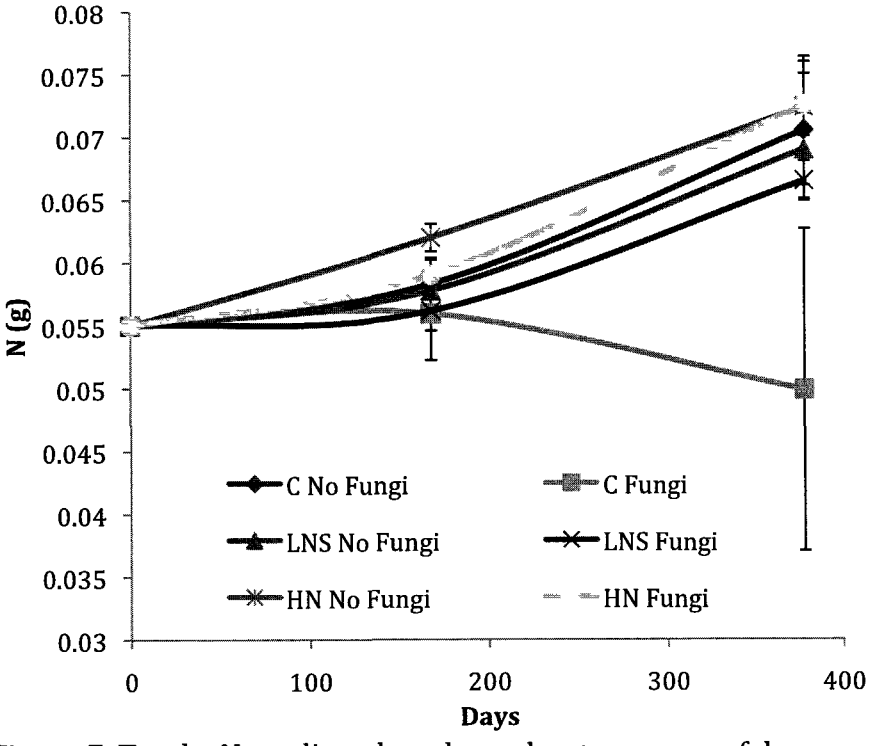
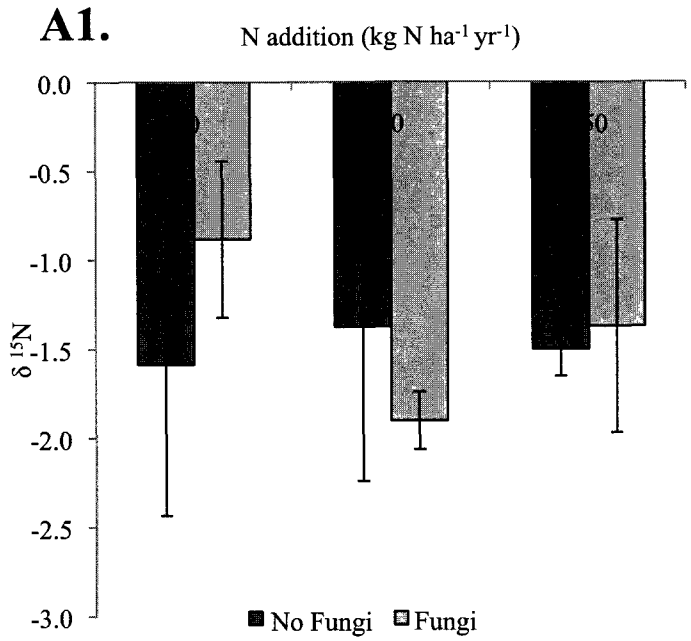
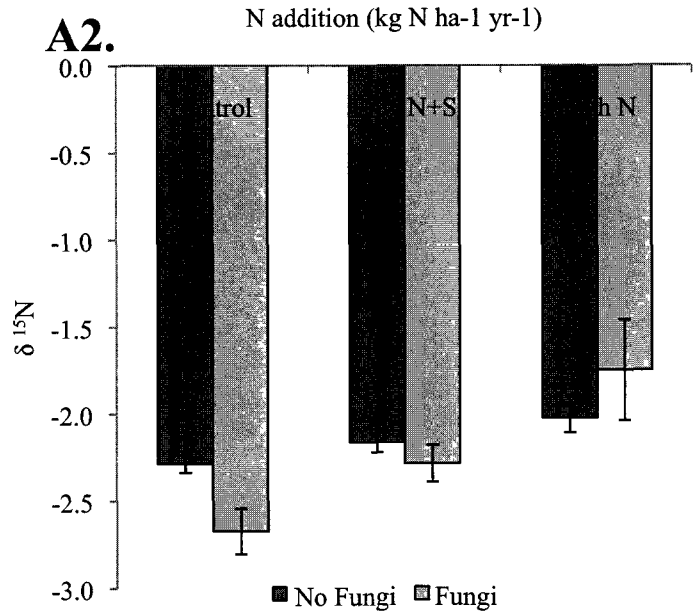


Figure 7. Total g N per litter bag throughout one year of decomposition at the Harvard Forest Chronic Nitrogen Amendment Study, Petersham, Ma. Points represent average (n = 8) from litter bags (no fungi and fungi) in control (C; no N additions), low N+S (LNS; 50 kg N ha^{-1} yr^{-1}) and high N (HN; 50 kg N ha^{-1} yr^{-1}).


A1.
N addition (kg N ha-1 yr-1)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
δ 15N
No Fungi
Fungi


A2.
N addition (kg N ha-1 yr-1)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
δ 15N
No Fungi
Fungi

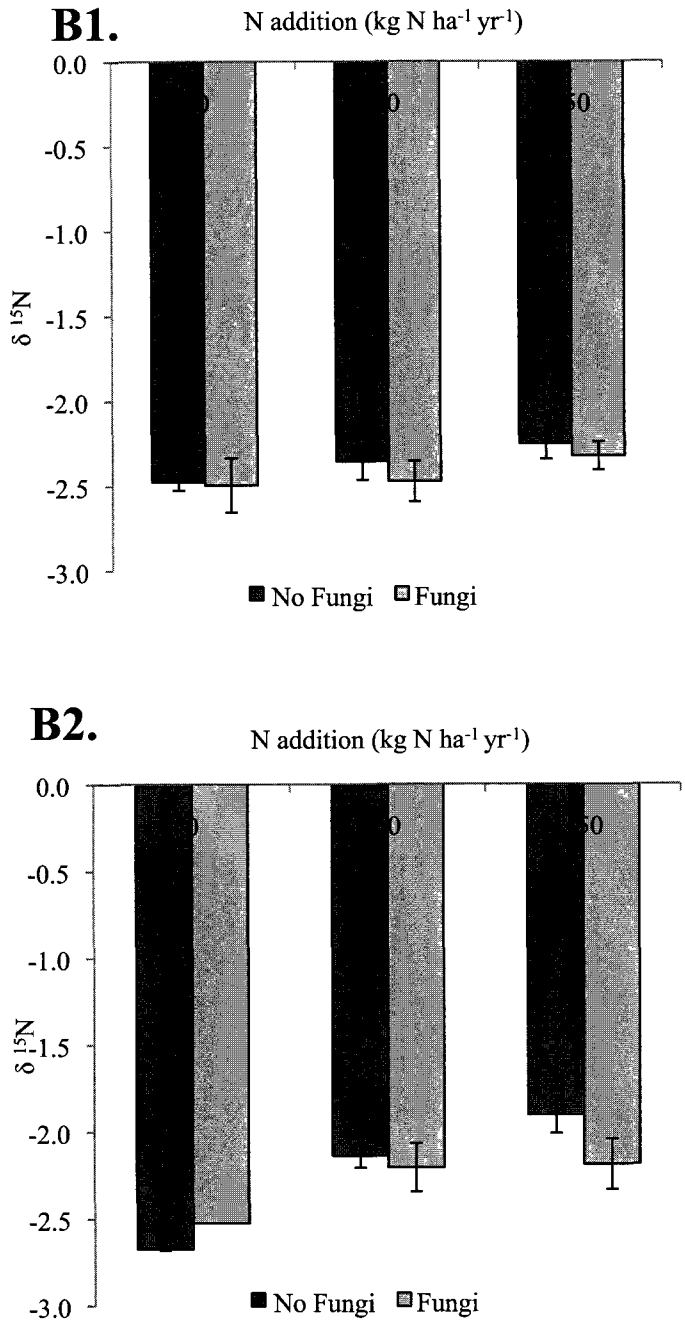


Figure 8. Leaf litter $\delta^{15}N$ values for enriched (A) and natural abundance (B) samples for 5 mo. (1) and 12 mo. (2) time points from control (C; no N additions), low N+S (LNS; 50 kg N ha^{-1} yr^{-1}) and high N (HN; 50 kg N ha^{-1} yr^{-1}) treatments. Columns represent means (n = 4), while error bars are one standard error of the mean. No fungi and fungi headings represent litter bag type and whether hyphal connections between the soil litter interface were restricted (no fungi) or allowed (fungi). Enriched samples had soil beneath litter bags labeled with a 98 at. % ^{15}N-$(NH_4)_2SO_4$ one week before sampling.

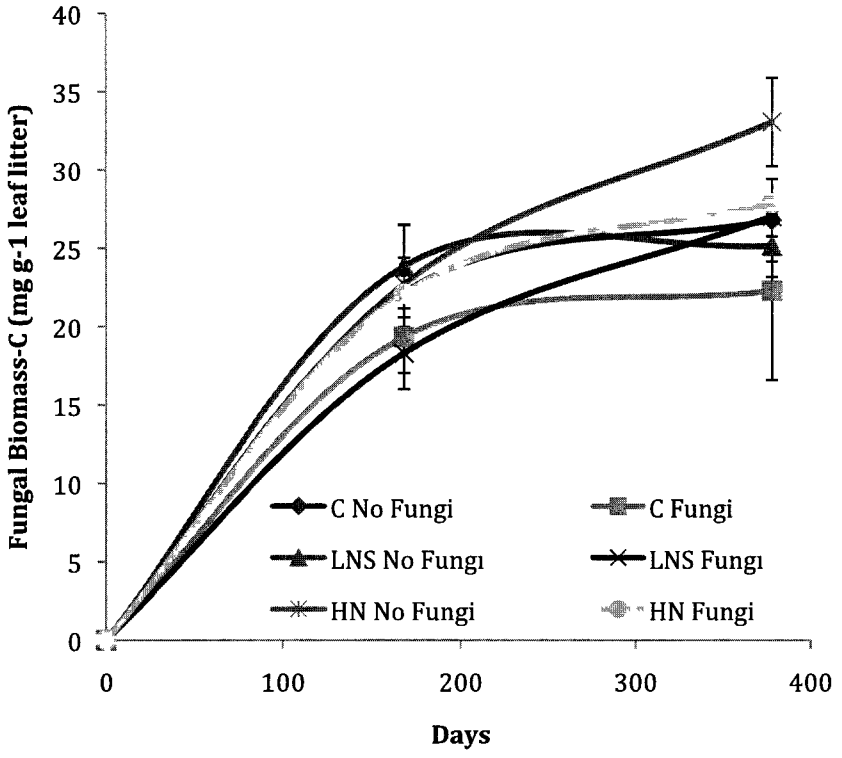


Figure 9. Fungal biomass-C (mg g^{-1} leaf litter) at 0, 5, and 12 months of decomposition at the Harvard Forest Chronic Nitrogen Amendment Experiment, Petersham, Ma. Values are averages (n=8) from litter bags (no fungi and fungi) in control (C; no N additions), low N+S (LNS; 50 kg N ha^{-1} yr^{-1}) and high N (HN; 50 kg N ha^{-1} yr^{-1}).

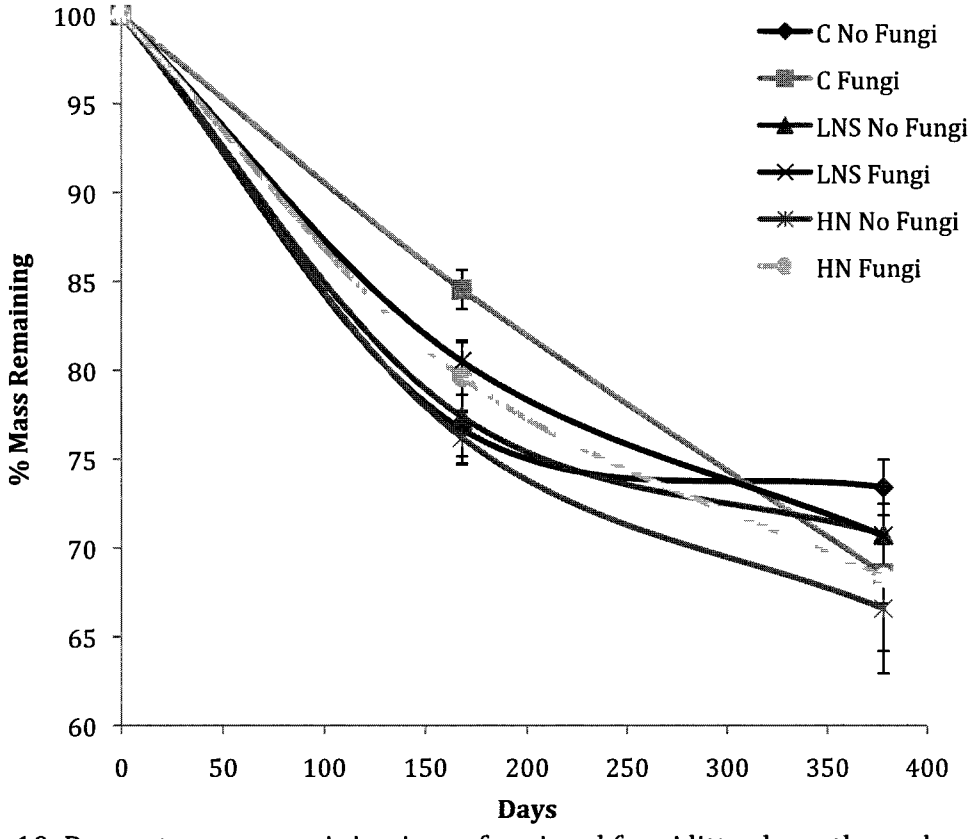


Figure 10. Percent mass remaining in no fungi and fungi litter bags throughout one year decomposition period from litter bags (no fungi and fungi) in control (C; no N additions), low N+S (LNS; 50 kg N ha^{-1} yr^{-1}) and high N (HN; 50 kg N ha^{-1} yr^{-1}).treatments at the Harvard Forest Chronic Nitrogen Amendment Experiment, Petersham, Ma.

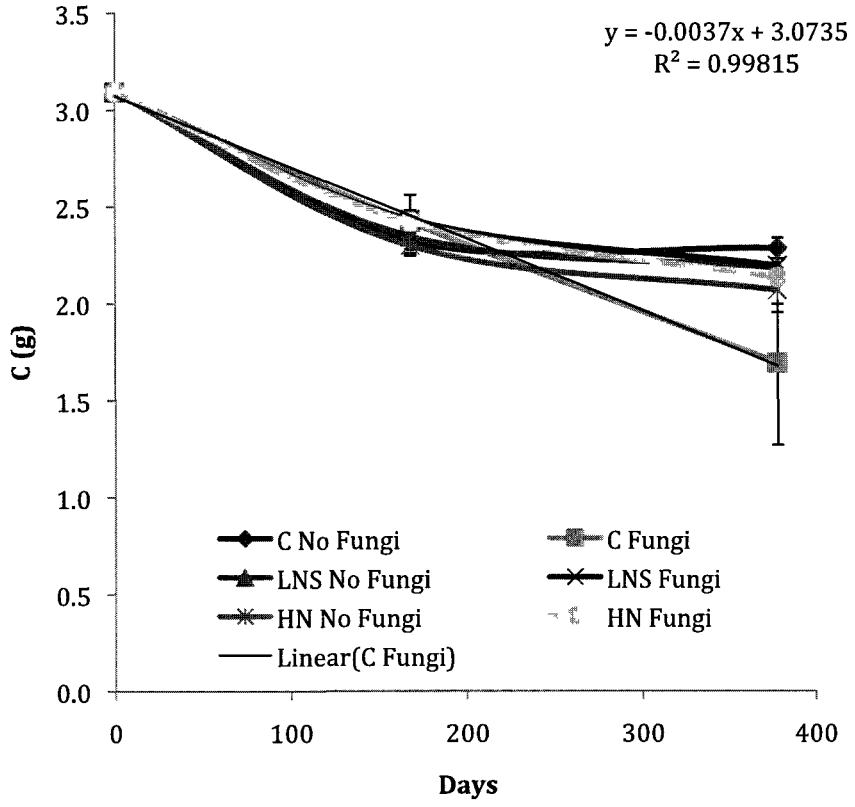


Figure 11. Total g C per litter bag throughout one year of decomposition at the Harvard Forest Chronic Nitrogen Amendment Study, Petersham, Ma. Points represent average (n = 8) from litter bags (no fungi and fungi) in control (C; no N additions), low N+S (LNS; 50 kg N ha^{-1} yr^{-1}) and high N (HN; 50 kg N ha^{-1} yr^{-1}

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DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Bulk Density (g cm^{-3})	Soil pH	Soil Moisture Content	Litter Moisture Content
10/28/10	no	0	C	R1-4	NOT	0.14	3.95	1.54	2.55
10/28/10	no	0	C	R1-4	T	0.10	3.69	1.44	1.18
10/28/10	no	0	C	R2-5	NOT	0.12	3.76	1.81	2.14
10/28/10	no	0	C	R2-5	T	0.16	3.85	1.67	0.85
10/28/10	no	0	C	R3-5	NOT	0.15	4.06	1.82	4.60
10/28/10	no	0	C	R3-5	T	0.13	3.95	2.17	2.25
10/28/10	no	0	C	R4-5	NOT	0.12	3.93	1.65	2.31
10/28/10	no	0	C	R4-5	T	0.13	3.77	1.85	1.27
10/28/10	no	50	LNS	C5-6	NOT	0.15	4.04	2.31	2.14
10/28/10	no	50	LNS	C5-6	T	0.12	3.91	2.28	0.99
10/28/10	no	50	LNS	D2-5	NOT	0.14	3.91	1.87	2.43
10/28/10	no	50	LNS	D2-5	T	0.12	3.93	2.19	1.84
10/28/10	no	50	LNS	E3-6	NOT	0.11	4.08	2.39	3.24
10/28/10	no	50	LNS	E3-6	T	0.11	4.17	2.70	1.72
10/28/10	no	50	LNS	E5-2	NOT	0.12	3.81	1.95	1.61
10/28/10	no	50	LNS	E5-2	T	0.13	3.87	2.10	1.34
10/28/10	no	150	HN	B3-3	NOT	0.09	3.86	2.22	2.14
10/28/10	no	150	HN	B5-3	NOT	0.14	4.27	2.41	0.87
10/28/10	no	150	HN	B5-3	T	0.10	4.08	2.42	1.47
10/28/10	no	150	HN	C2-6	NOT	0.10	4.12	1.88	1.94
10/28/10	no	150	HN	C2-6	T	0.10	4.12	2.05	2.04
10/28/10	no	150	HN	E4-5	NOT	0.24	3.86	0.89	1.19
10/28/10	no	150	HN	E4-5	T	0.16	3.78	1.51	1.09

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	NH4-N (KCl) ug g^{-1} soil	NO3-N (KCl) ug g^{-1} soil	NH4-N (H_2O) ug g^{-1} soil	NO3-N (H_2O) ug g^{-1} soil
10/28/10	no	0	C	R1-4	NOT	2.94	0.00		
10/28/10	no	0	C	R1-4	T	1.56	0.00		
10/28/10	no	0	C	R2-5	NOT	0.63	0.00		
10/28/10	no	0	C	R2-5	T	0.15	0.00		
10/28/10	no	0	C	R3-5	NOT	2.59	0.00		
10/28/10	no	0	C	R3-5	T	2.04	0.00		
10/28/10	no	0	C	R4-5	NOT	2.06	0.00		
10/28/10	no	0	C	R4-5	T	4.00	0.00		
10/28/10	no	50	LNS	C5-6	NOT	2.16	0.00		
10/28/10	no	50	LNS	C5-6	T	2.77	0.00		
10/28/10	no	50	LNS	D2-5	NOT	0.97	0.00		
10/28/10	no	50	LNS	D2-5	T	3.58	0.00		
10/28/10	no	50	LNS	E3-6	NOT	2.44	0.00		
10/28/10	no	50	LNS	E3-6	T	4.98	0.00		
10/28/10	no	50	LNS	E5-2	NOT	5.00	0.00		
10/28/10	no	50	LNS	E5-2	T	6.88	0.00		
10/28/10	no	150	HN	B3-3	NOT	36.06	0.00		
10/28/10	no	150	HN	B5-3	NOT	31.18	0.00		
10/28/10	no	150	HN	B5-3	T	25.12	0.00		
10/28/10	no	150	HN	C2-6	NOT	27.80	0.00		
10/28/10	no	150	HN	C2-6	T	16.39	0.00		
10/28/10	no	150	HN	E4-5	NOT	12.23	0.00		
10/28/10	no	150	HN	E4-5	T	15.64	0.00		

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Litter Dry Mass 5 or 12 mo. (g)	Litter Dry Mass 0 mo. (g)	Litter Mass Lost (g)	% Litter Mass Remaining
10/28/10	no	0	C	R1-4	NOT	0.73	6	*bag ripped	
10/28/10	no	0	C	R1-4	T	2.94	5.99	*bag ripped	
10/28/10	no	0	C	R2-5	NOT	4.65	6.03	1.38	77.12
10/28/10	no	0	C	R2-5	T	5.33	6	0.67	88.86
10/28/10	no	0	C	R3-5	NOT	4.35	6.04	1.69	71.94
10/28/10	no	0	C	R3-5	T	4.82	6.03	1.21	79.94
10/28/10	no	0	C	R4-5	NOT	4.78	6.03	1.25	79.31
10/28/10	no	0	C	R4-5	T	5.08	6.01	0.93	84.55
10/28/10	no	50	LNS	C5-6	NOT	4.65	6.02	1.37	77.30
10/28/10	no	50	LNS	C5-6	T	5.16	6.04	0.88	85.45
10/28/10	no	50	LNS	D2-5	NOT	4.54	5.98	1.44	75.87
10/28/10	no	50	LNS	D2-5	T	4.64	6	1.36	77.41
10/28/10	no	50	LNS	E3-6	NOT	4.35	5.97	1.62	72.87
10/28/10	no	50	LNS	E3-6	T	4.83	6.04	1.21	80.04
10/28/10	no	50	LNS	E5-2	NOT	5.06	6.02	0.96	84.11
10/28/10	no	50	LNS	E5-2	T	5.00	5.99	0.99	83.54
10/28/10	no	150	HN	B3-3	NOT	4.33	6	1.67	72.10
10/28/10	no	150	HN	B5-3	NOT	4.81	5.99	1.18	80.23
10/28/10	no	150	HN	B5-3	T	4.74	5.98	1.24	79.26
10/28/10	no	150	HN	C2-6	NOT	4.52	5.97	1.45	75.72
10/28/10	no	150	HN	C2-6	T	4.78	5.98	1.20	79.94
10/28/10	no	150	HN	E4-5	NOT	4.88	6.01	1.13	81.27
10/28/10	no	150	HN	E4-5	T	4.89	6	1.11	81.46

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	^{15}N	%N	^{13}C	%C	C:N	Total N (g bag^{-1})	N gained (g bag^{-1})	Total C (g bag^{-1})
10/28/10	no	0	C	R1-4	NOT								
10/28/10	no	0	C	R1-4	T	-2.65	1.12	-28.95	49.71	44.20	0.033	-0.022	1.46
10/28/10	no	0	C	R2-5	NOT	-2.40	1.46	-28.88	50.56	34.56	0.068	0.013	2.35
10/28/10	no	0	C	R2-5	T	-2.30	1.16	-28.72	50.49	43.56	0.062	0.007	2.69
10/28/10	no	0	C	R3-5	NOT	-2.57	1.37	-28.84	50.39	36.69	0.060	0.005	2.19
10/28/10	no	0	C	R3-5	T	-2.87	1.12	-28.91	49.76	44.47	0.054	-0.001	2.40
10/28/10	no	0	C	R4-5	NOT	-2.47	1.14	-28.75	50.44	44.35	0.054	-0.001	2.41
10/28/10	no	0	C	R4-5	T	-2.17	1.03	-28.61	51.03	49.52	0.052	-0.003	2.59
10/28/10	no	50	LNS	C5-6	NOT	-2.29	1.27	-28.74	50.77	39.98	0.059	0.004	2.36
10/28/10	no	50	LNS	C5-6	T	-2.36	0.94	-31.12	48.18	51.14	0.049	-0.006	2.49
10/28/10	no	50	LNS	D2-5	NOT	-2.49	1.23	-29.28	50.29	40.92	0.056	0.001	2.28
10/28/10	no	50	LNS	D2-5	T	-2.35	1.22	-28.92	50.71	41.52	0.057	0.002	2.36
10/28/10	no	50	LNS	E3-6	NOT	-2.57	1.12	-29.19	49.72	44.37	0.049	-0.006	2.16
10/28/10	no	50	LNS	E3-6	T	-2.83	1.12	-28.95	50.26	44.92	0.054	-0.001	2.43
10/28/10	no	50	LNS	E5-2	NOT	-2.09	1.13	-28.86	50.87	44.89	0.057	0.002	2.58
10/28/10	no	50	LNS	E5-2	T	-2.35	1.06	-28.74	50.76	47.68	0.053	-0.002	2.54
10/28/10	no	150	HN	B3-3	NOT	-2.23	1.56	-28.64	50.59	32.37	0.068	0.013	2.19
10/28/10	no	150	HN	B5-3	NOT	-2.43	1.34	-28.97	50.23	37.58	0.064	0.009	2.41
10/28/10	no	150	HN	B5-3	T	-2.39	1.21	-29.44	50.95	42.13	0.057	0.002	2.41
10/28/10	no	150	HN	C2-6	NOT	-2.01	1.40	-28.18	49.83	35.68	0.063	0.008	2.25
10/28/10	no	150	HN	C2-6	T	-2.16	1.11	-28.55	50.83	45.74	0.053	-0.002	2.43
10/28/10	no	150	HN	E4-5	NOT	-2.34	1.17	-28.99	50.44	43.04	0.057	0.002	2.46
10/28/10	no	150	HN	E4-5	T	-2.42	1.29	-29.37	50.27	38.85	0.063	0.008	2.46

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	C lost (g bag^{-1})	Ergosterol (ug g^{-1} litter)	Fungal Biomass-C (mg g^{-1} litter)	Fungal Biomass-C (mg bag^{-1})	Fungal Biomass-C (% of bag)
10/28/10	no	0	C	R1-4	NOT		275.1	24.76	34.10	2.48
10/28/10	no	0	C	R1-4	T	1.63	265.3	23.88	70.22	2.39
10/28/10	no	0	C	R2-5	NOT	0.74	263.6	23.73	110.33	2.37
10/28/10	no	0	C	R2-5	T	0.40	224.1	20.17	107.54	2.02
10/28/10	no	0	C	R3-5	NOT	0.90	141.8	12.76	55.45	1.28
10/28/10	no	0	C	R3-5	T	0.69	233.1	20.98	101.12	2.10
10/28/10	no	0	C	R4-5	NOT	0.68	320.1	28.81	137.79	2.88
10/28/10	no	0	C	R4-5	T	0.50	119.0	10.71	54.43	1.07
10/28/10	no	50	LNS	C5-6	NOT	0.73	321.4	28.93	134.61	2.89
10/28/10	no	50	LNS	C5-6	T	0.60	220.5	19.84	102.41	1.98
10/28/10	no	50	LNS	D2-5	NOT	0.81	305.9	27.53	124.91	2.75
10/28/10	no	50	LNS	D2-5	T	0.73	212.4	19.11	88.78	1.91
10/28/10	no	50	LNS	E3-6	NOT	0.93	267.4	24.06	104.69	2.41
10/28/10	no	50	LNS	E3-6	T	0.66	102.7	9.24	44.67	0.92
10/28/10	no	50	LNS	E5-2	NOT	0.51	231.4	20.83	105.46	2.08
10/28/10	no	50	LNS	E5-2	T	0.55	94.8	8.53	42.70	0.85
10/28/10	no	150	HN	B3-3	NOT	0.90	385.1	34.66	149.93	3.47
10/28/10	no	150	HN	B5-3	NOT	0.68			0.00	0.00
10/28/10	no	150	HN	B5-3	T	0.68	347.2	31.25	148.10	3.12
10/28/10	no	150	HN	C2-6	NOT	0.84	291.2	26.21	118.47	2.62
10/28/10	no	150	HN	C2-6	T	0.66	81.5	7.34	35.07	0.73
10/28/10	no	150	HN	E4-5	NOT	0.63	21.8	1.96	9.59	0.20
10/28/10	no	150	HN	E4-5	T	0.63	267.8	24.11	117.82	2.41

DATE	^{15}NEnrichment	N addition(kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Bulk Density(g cm^{-3})	Soil pH	Soil MoistureContent	Litter Moisture Content
11/04/10	yes	0	C	R1-5	NOT		4.47	0.83	2.15
11/04/10	yes	0	C	R1-5	T		4	0.98	1.42
11/04/10	yes	0	C	R2-2	NOT		4.02	2.20	3.19
11/04/10	yes	0	C	R2-2	T		4.28	0.98	1.38
11/04/10	yes	0	C	R3-2	NOT		3.99	2.46	2.81
11/04/10	yes	0	C	R3-2	T		3.93	2.07	1.29
11/04/10	yes	0	C	R4-6	NOT		3.86	2.99	2.54
11/04/10	yes	0	C	R4-6	T		3.74	1.84	1.62
11/04/10	yes	50	LNS	C5-6 *	NOT		3.99	2.13	3.12
11/04/10	yes	50	LNS	C5-6 *	T		4.01	2.46	1.53
11/04/10	yes	50	LNS	D2-3	NOT		4.2	2.86	3.04
11/04/10	yes	50	LNS	D2-3	T		3.98	1.61	1.66
11/04/10	yes	50	LNS	E3-4	NOT		4.37	3.42	3.97
11/04/10	yes	50	LNS	E3-4	T		4.33	2.99	2.14
11/04/10	yes	50	LNS	E5-3	NOT		4.59	0.49	1.45
11/04/10	yes	50	LNS	E5-3	T		4.41	0.96	1.76
11/04/10	yes	150	HN	B3-2	NOT		4.13	2.86	3.43
11/04/10	yes	150	HN	B3-2	T		4.09	2.70	1.27
11/04/10	yes	150	HN	B5-5	NOT		4.3	3.37	3.86
11/04/10	yes	150	HN	B5-5	T		4.49	3.51	2.68
11/04/10	yes	150	HN	C2-4	NOT		4.17	2.15	2.42
11/04/10	yes	150	HN	C2-4	T		4.17	3.34	2.61
11/04/10	yes	150	HN	E4-1	NOT		3.6	1.75	1.69
11/04/10	yes	150	HN	E4-1	T		3.9	1.64	1.14

DATE	^{15}NEnrichment	N addition(kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	NH4-N (KCl)ug g^{-1} soil	NO3-N (KCl)ug g^{-1} soil	NH4-N (H_2O)ug g^{-1} soil	NO3-N (H_2O)ug g^{-1} soil
11/04/10	yes	0	C	R1-5	NOT	1.90	0.00		
11/04/10	yes	0	C	R1-5	T	0.52	0.00		
11/04/10	yes	0	C	R2-2	NOT	3.17	0.00		
11/04/10	yes	0	C	R2-2	T	6.58	0.00		
11/04/10	yes	0	C	R3-2	NOT	11.29	0.00		
11/04/10	yes	0	C	R3-2	T	4.00	0.00		
11/04/10	yes	0	C	R4-6	NOT	2.77	0.00		
11/04/10	yes	0	C	R4-6	T	4.31	0.00		
11/04/10	yes	50	LNS	C5-6 *	NOT	14.91	0.00		
11/04/10	yes	50	LNS	C5-6 *	T	11.55	0.00		
11/04/10	yes	50	LNS	D2-3	NOT	4.50	0.00		
11/04/10	yes	50	LNS	D2-3	T	3.47	0.00		
11/04/10	yes	50	LNS	E3-4	NOT	13.98	0.00		
11/04/10	yes	50	LNS	E3-4	T	7.18	0.00		
11/04/10	yes	50	LNS	E5-3	NOT	0.51	0.00		
11/04/10	yes	50	LNS	E5-3	T	4.00	0.00		
11/04/10	yes	150	HN	B3-2	NOT	37.96	0.00		
11/04/10	yes	150	HN	B3-2	T	32.89	0.00		
11/04/10	yes	150	HN	B5-5	NOT	40.42	0.00		
11/04/10	yes	150	HN	B5-5	T	55.06	0.00		
11/04/10	yes	150	HN	C2-4	NOT	20.76	0.00		
11/04/10	yes	150	HN	C2-4	T	29.00	0.00		
11/04/10	yes	150	HN	E4-1	NOT	26.04	0.00		
11/04/10	yes	150	HN	E4-1	T	27.32	0.00		

DATE	^{15}NEnrichment	N addition(kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Litter Dry Mass5 or 12 mo. (g)	Litter Dry Mass0 mo. (g)	Litter MassLost (g)	% Litter MassRemaining
11/04/10	yes	0	C	R1-5	NOT	4.81	6.02	1.21	79.88
11/04/10	yes	0	C	R1-5	T	5.19	6	0.81	86.51
11/04/10	yes	0	C	R2-2	NOT	4.04	6	1.96	67.36
11/04/10	yes	0	C	R2-2	T	4.90	5.98	1.08	81.98
11/04/10	yes	0	C	R3-2	NOT	4.84	5.99	1.15	80.72
11/04/10	yes	0	C	R3-2	T	5.09	6.03	0.94	84.37
11/04/10	yes	0	C	R4-6	NOT	4.81	5.97	1.16	80.49
11/04/10	yes	0	C	R4-6	T	5.13	6	0.87	85.51
11/04/10	yes	50	LNS	C5-6 *	NOT	4.58	6	1.42	76.40
11/04/10	yes	50	LNS	C5-6 *	T	4.84	5.99	1.15	80.75
11/04/10	yes	50	LNS	D2-3	NOT	4.80	5.99	1.19	80.06
11/04/10	yes	50	LNS	D2-3	T	5.00	6.01	1.01	83.17
11/04/10	yes	50	LNS	E3-4	NOT	3.96	5.99	2.03	66.16
11/04/10	yes	50	LNS	E3-4	T	4.60	6.03	1.43	76.31
11/04/10	yes	50	LNS	E5-3	NOT	5.19	6.01	0.82	86.29
11/04/10	yes	50	LNS	E5-3	T	4.64	6	1.36	77.28
11/04/10	yes	150	HN	B3-2	NOT	4.74	6	1.26	78.97
11/04/10	yes	150	HN	B3-2	T	5.08	6	0.92	84.61
11/04/10	yes	150	HN	B5-5	NOT	4.49	6	1.51	74.90
11/04/10	yes	150	HN	B5-5	T	4.37	6	1.63	72.85
11/04/10	yes	150	HN	C2-4	NOT	4.11	5.99	1.88	68.67
11/04/10	yes	150	HN	C2-4	T	4.39	6.01	1.62	73.00
11/04/10	yes	150	HN	E4-1	NOT	4.68	6.02	1.34	77.72
11/04/10	yes	150	HN	E4-1	T	5.21	6.05	0.84	86.20

DATE	^{15}NEnrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	^{15}N	%N	^{13}C	%C	C:N	Total N(g bag^{-1})	N gained (g bag^{-1})	Total C (g bag^{-1})
11/04/10	yes	0	C	R1-5	NOT	-2.46	1.16	-28.67	50.45	43.33	0.056	0.001	2.43
11/04/10	yes	0	C	R1-5	T	-1.59	1.11	-28.76	50.85	45.77	0.058	0.003	2.64
11/04/10	yes	0	C	R2-2	NOT	0.95	1.29	-28.66	49.25	38.05	0.052	-0.003	1.99
11/04/10	yes	0	C	R2-2	T	0.26	1.18	-28.72	49.67	41.93	0.058	0.003	2.43
11/04/10	yes	0	C	R3-2	NOT	-2.36	1.25	-29.21	50.80	40.63	0.060	0.005	2.46
11/04/10	yes	0	C	R3-2	T	-1.57	1.24	-28.96	49.98	40.30	0.063	0.008	2.54
11/04/10	yes	0	C	R4-6	NOT	-2.48	1.19	-29.37	50.23	42.32	0.057	0.002	2.41
11/04/10	yes	0	C	R4-6	T	-0.64	1.31	-29.45	50.26	38.28	0.067	0.012	2.58
11/04/10	yes	50	LNS	C5-6	NOT	-0.51	1.36	-29.17	50.89	37.44	0.062	0.007	2.33
11/04/10	yes	50	LNS	C5-6	T	-1.51	1.28	-28.97	50.84	39.78	0.062	0.007	2.46
11/04/10	yes	50	LNS	D2-3	NOT	-2.24	1.24	-28.85	51.20	41.40	0.059	0.004	2.46
11/04/10	yes	50	LNS	D2-3	T	-2.25	1.24	-29.07	51.24	41.20	0.062	0.007	2.56
11/04/10	yes	50	LNS	E3-4	NOT		1.51	-29.52	50.45	33.51	0.060	0.005	2.00
11/04/10	yes	50	LNS	E3-4	T	-1.80	1.21	-28.51	50.76	41.80	0.056	0.001	2.34
11/04/10	yes	50	LNS	E5-3	NOT		1.15	-28.74	49.79	43.21	0.060	0.005	2.58
11/04/10	yes	50	LNS	E5-3	T	-2.06	1.22	-28.96	49.05	40.23	0.057	0.002	2.27
11/04/10	yes	150	HN	B3-2	NOT	-1.09	1.27	-28.83	50.38	39.69	0.060	0.005	2.39
11/04/10	yes	150	HN	B3-2	T	0.41	1.12	-29.44	49.96	44.77	0.057	0.002	2.54
11/04/10	yes	150	HN	B5-5	NOT	-1.51	1.38	-28.67	51.24	37.25	0.062	0.007	2.30
11/04/10	yes	150	HN	B5-5	T	-2.00	1.41	-29.25	51.26	36.45	0.061	0.006	2.24
11/04/10	yes	150	HN	C2-4	NOT	-1.58	1.48	-28.95	48.79	33.05	0.061	0.006	2.01
11/04/10	yes	150	HN	C2-4	T	-1.81	1.35	-28.89	50.31	37.27	0.059	0.004	2.21
11/04/10	yes	150	HN	E4-1	NOT	-1.82	1.30	-28.36	50.37	38.79	0.061	0.006	2.36
11/04/10	yes	150	HN	E4-1	T	-2.09	1.19	-29.54	50.85	42.82	0.062	0.007	2.65

DATE	^{15}NEnrichment	N addition(kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	C lost(g bag^{-1})	Ergosterol(ug g^{-1} litter)	FungalBiomass-C(mg g^{-1} litter)	FungalBiomass-C(mg bag^{-1})	FungalBiomass-C(% of bag)
11/04/10	yes	0	C	R1-5	NOT	0.66	303.0	27.27	131.15	2.73
11/04/10	yes	0	C	R1-5	T	0.45			0.00	0.00
11/04/10	yes	0	C	R2-2	NOT	1.10			0.00	0.00
11/04/10	yes	0	C	R2-2	T	0.66	316.0	28.44	139.40	2.84
11/04/10	yes	0	C	R3-2	NOT	0.63	177.4	15.96	77.19	1.60
11/04/10	yes	0	C	R3-2	T	0.55	200.4	18.04	91.78	1.80
11/04/10	yes	0	C	R4-6	NOT	0.68	244.2	21.98	105.61	2.20
11/04/10	yes	0	C	R4-6	T	0.51	145.6	13.10	67.23	1.31
11/04/10	yes	50	LNS	C5-6 *	NOT	0.76	90.9	8.18	37.50	0.82
11/04/10	yes	50	LNS	C5-6 *	T	0.63	315.0	28.35	137.12	2.83
11/04/10	yes	50	LNS	D2-3	NOT	0.63	374.3	33.69	161.55	3.37
11/04/10	yes	50	LNS	D2-3	T	0.53	220.0	19.80	98.98	1.98
11/04/10	yes	50	LNS	E3-4	NOT	1.09	291.2	26.21	103.86	2.62
11/04/10	yes	50	LNS	E3-4	T	0.75	228.1	20.53	94.47	2.05
11/04/10	yes	50	LNS	E5-3	NOT	0.51	236.4	21.28	110.35	2.13
11/04/10	yes	50	LNS	E5-3	T	0.82	232.8	20.95	97.15	2.10
11/04/10	yes	150	HN	B3-2	NOT	0.70	283.3	25.50	120.80	2.55
11/04/10	yes	150	HN	B3-2	T	0.55	260.5	23.44	119.01	2.34
11/04/10	yes	150	HN	B5-5	NOT	0.79	255.6	23.01	103.40	2.30
11/04/10	yes	150	HN	B5-5	T	0.85	409.6	36.86	161.12	3.69
11/04/10	yes	150	HN	C2-4	NOT	1.08	263.6	23.73	97.59	2.37
11/04/10	yes	150	HN	C2-4	T	0.88			0.00	0.00
11/04/10	yes	150	HN	E4-1	NOT	0.73	265.3	23.87	111.70	2.39
11/04/10	yes	150	HN	E4-1	T	0.44	106.6	9.60	50.05	0.96

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Bulk Density (g cm^{-3})	Soil pH	Soil Moisture Content	Litter Moisture Content
5/26/11	no	0	C	R1-3	NOT			0.93	0.75
5/26/11	no	0	C	R1-3	T			0.97	0.26
5/26/11	no	0	C	R2-1	NOT			0.96	0.30
5/26/11	no	0	C	R2-1	T			0.78	
5/26/11	no	50	LNS	C5-1	NOT			1.61	0.56
5/26/11	no	50	LNS	C5-1	T			1.17	0.33
5/26/11	no	50	LNS	E5-4	NOT			2.12	0.11
5/26/11	no	50	LNS	E5-4	T			2.22	0.84
5/26/11	no	150	HN	B5-6	NOT			2.67	1.20
5/26/11	no	150	HN	B5-6	T			2.55	0.99
5/26/11	no	150	HN	E4-3	NOT			1.66	2.88
5/26/11	no	150	HN	E4-3	T			2.60	2.38

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	NH4-N (KCl) ug g^{-1} soil	NO3-N (KCl) ug g^{-1} soil	NH4-N (H_2O) ug g^{-1} soil	NO3-N (H_2O) ug g^{-1} soil
5/26/11	no	0	C	R1-3	NOT	20.88	0.00	6.96	-0.50
5/26/11	no	0	C	R1-3	T	21.72	0.00	5.52	-0.39
5/26/11	no	0	C	R2-1	NOT	15.77	0.00	6.39	2.09
5/26/11	no	0	C	R2-1	T	13.92	0.00	3.67	-0.59
5/26/11	no	50	LNS	C5-1	NOT	51.35	0.00	11.27	-0.88
5/26/11	no	50	LNS	C5-1	T	35.95	0.00	11.86	-0.77
5/26/11	no	50	LNS	E5-4	NOT	48.83	0.00	16.08	-0.88
5/26/11	no	50	LNS	E5-4	T	30.66	0.00	19.04	-0.34
5/26/11	no	150	HN	B5-6	NOT	44.78	0.00	17.61	1.95
5/26/11	no	150	HN	B5-6	T	62.19	0.00	22.45	1.53
5/26/11	no	150	HN	E4-3	NOT	64.25	0.00	54.08	-0.85
5/26/11	no	150	HN	E4-3	T	76.95	1.10	58.23	-1.42

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Litter Dry Mass 5 or 12 mo. (g)	Litter Dry Mass 0 mo. (g)	Litter Mass Lost (g)	% Litter Mass Remaining
5/26/11	no	0	C	R1-3	NOT	4.24	5.98	1.74	70.89
5/26/11	no	0	C	R1-3	T	4.21	6	1.79	70.20
5/26/11	no	0	C	R2-1	NOT	4.17	5.99	1.82	69.57
5/26/11	no	0	C	R2-1	T				
5/26/11	no	50	LNS	C5-1	NOT	4.46	5.99	1.53	74.53
5/26/11	no	50	LNS	C5-1	T	4.55	6	1.45	75.80
5/26/11	no	50	LNS	E5-4	NOT	4.27	6	1.73	71.14
5/26/11	no	50	LNS	E5-4	T	3.78	5.94	2.16	63.65
5/26/11	no	150	HN	B5-6	NOT	4.12	6.01	1.89	68.61
5/26/11	no	150	HN	B5-6	T	4.32	6	1.68	72.03
5/26/11	no	150	HN	E4-3	NOT	3.51	6.01	2.50	58.36
5/26/11	no	150	HN	E4-3	T	3.67	5.99	2.32	61.25

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	^{15}N	%N	^{13}C	%C	C:N	Total N (g bag^{-1})	N gained (g bag^{-1})	Total C (g bag^{-1})
5/26/11	no	0	C	R1-3	NOT	-2.67	1.52	-29.28	51.50	33.94	0.064	0.009	2.183
5/26/11	no	0	C	R1-3	T	-2.53	1.45	-29.20	50.49	34.78	0.061	0.006	2.127
5/26/11	no	0	C	R2-1	NOT	-2.68	1.56	-29.13	51.96	33.40	0.065	0.010	2.165
5/26/11	no	0	C	R2-1	T								
5/26/11	no	50	LNS	C5-1	NOT	-2.34	1.68	-29.15	52.01	30.99	0.075	0.020	2.322
5/26/11	no	50	LNS	C5-1	T	-2.10	1.41	-29.50	52.39	37.07	0.064	0.009	2.383
5/26/11	no	50	LNS	E5-4	NOT	-2.03	1.72	-28.62	50.19	29.26	0.073	0.018	2.142
5/26/11	no	50	LNS	E5-4	T	-2.19	1.98	-28.75	51.02	25.74	0.075	0.020	1.929
5/26/11	no	150	HN	B5-6	NOT	-1.61	1.62	-29.48	51.91	32.11	0.067	0.012	2.140
5/26/11	no	150	HN	B5-6	T	-2.08	1.54	-29.72	51.34	33.40	0.066	0.011	2.219
5/26/11	no	150	HN	E4-3	NOT	-1.89	1.69	-29.67	52.00	30.71	0.059	0.004	1.824
5/26/11	no	150	HN	E4-3	T	-2.04	1.77	-28.88	51.54	29.15	0.065	0.010	1.891

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	C lost (g bag^{-1})	Ergosterol (ug g^{-1} litter)	Fungal Biomass-C (mg g^{-1} litter)	Fungal Biomass-C (mg bag^{-1})	Fungal Biomass-C (% of bag)
5/26/11	no	0	C	R1-3	NOT	0.907	285.0	25.65	108.73	2.56
5/26/11	no	0	C	R1-3	T	0.963	307.1	27.64	116.40	2.76
5/26/11	no	0	C	R2-1	NOT	0.925	298.6	26.88	112.00	2.69
5/26/11	no	0	C	R2-1	T					
5/26/11	no	50	LNS	C5-1	NOT	0.768	331.8	29.86	133.32	2.99
5/26/11	no	50	LNS	C5-1	T	0.707	304.3	27.39	124.55	2.74
5/26/11	no	50	LNS	E5-4	NOT	0.948	376.1	33.85	144.50	3.39
5/26/11	no	50	LNS	E5-4	T	1.161	258.4	23.26	87.94	2.33
5/26/11	no	150	HN	B5-6	NOT	0.950	430.1	38.70	159.59	3.87
5/26/11	no	150	HN	B5-6	T	0.871	390.3	35.13	151.83	3.51
5/26/11	no	150	HN	E4-3	NOT	1.266	395.3	35.57	124.77	3.56
5/26/11	no	150	HN	E4-3	T	1.199	338.2	30.44	111.67	3.04

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Bulk Density (g cm^{-3})	Soil pH	Soil Moisture Content	Litter Moisture Content
6/2/11	yes	0	C	R1-6	NOT			1.25	0.76
6/2/11	yes	0	C	R1-6	T			1.20	0.42
6/2/11	yes	0	C	R2-4	NOT			1.27	0.35
6/2/11	yes	0	C	R2-4	T			1.14	0.60
6/2/11	yes	0	C	R4-3	NOT			0.81	0.29
6/2/11	yes	0	C	R4-3	T			0.70	0.33
6/2/11	yes	50	LNS	C5-3	NOT			2.28	0.68
6/2/11	yes	50	LNS	C5-3	T			1.64	0.56
6/2/11	yes	50	LNS	D2-1	NOT			2.37	0.85
6/2/11	yes	50	LNS	D2-1	T			2.14	0.58
6/2/11	yes	50	LNS	E3-5	NOT			2.13	0.32
6/2/11	yes	50	LNS	E3-5	T			2.08	0.75
6/2/11	yes	50	LNS	E5-5	NOT			1.47	0.18
6/2/11	yes	50	LNS	E5-5	T			1.66	0.30
6/2/11	yes	150	HN	B3-5	NOT			2.17	0.23
6/2/11	yes	150	HN	B3-5	T			2.15	0.31
6/2/11	yes	150	HN	B5-2	NOT			2.29	0.42
6/2/11	yes	150	HN	B5-2	T			2.26	0.25
6/2/11	yes	150	HN	C2-1	NOT			1.51	1.31
6/2/11	yes	150	HN	C2-1	T			2.07	0.66
6/2/11	yes	150	HN	E4-4	NOT			2.17	2.27
6/2/11	yes	150	HN	E4-4	T			2.31	0.98
6/2/11	no	50	LNS	D2-4	NOT			1.81	0.37
6/2/11	no	50	LNS	D2-4	T			2.12	0.21
6/2/11	no	50	LNS	E5-6	NOT			1.47	0.28

DATE	15N Enrichment	N addition (kg N ha-1 yr-1)	Plot	Field ID	NOT/T	NH4-N (KCl) ug g-1 soil	NO3-N (KCl) ug g-1 soil	NH4-N (H2O) ug g-1 soil	NO3-N (H2O) ug g-1 soil
6/2/11	yes	0	C	R1-6	NOT	12.72	0.86	8.08	-0.50
6/2/11	yes	0	C	R1-6	T	5.80	0.00	8.12	-0.15
6/2/11	yes	0	C	R2-4	NOT	6.53	0.00	6.57	0.43
6/2/11	yes	0	C	R2-4	T	6.50	0.00	9.89	-0.64
6/2/11	yes	0	C	R4-3	NOT	19.57	0.00	3.56	-0.33
6/2/11	yes	0	C	R4-3	T	17.72	0.00	10.74	-0.50
6/2/11	yes	50	LNS	C5-3	NOT	25.85	0.00	13.54	2.47
6/2/11	yes	50	LNS	C5-3	T	7.91	0.00	8.31	-0.89
6/2/11	yes	50	LNS	D2-1	NOT	14.88	0.00	12.35	-1.22
6/2/11	yes	50	LNS	D2-1	T	66.36	0.00	30.74	-1.19
6/2/11	yes	50	LNS	E3-5	NOT	17.90	0.00	21.78	-1.39
6/2/11	yes	50	LNS	E3-5	T	37.76	0.00	11.25	-1.05
6/2/11	yes	50	LNS	E5-5	NOT	20.85	0.00	13.55	-0.46
6/2/11	yes	50	LNS	E5-5	T	37.33	0.00	14.73	-0.41
6/2/11	yes	150	HN	B3-5	NOT	85.76	0.20	39.35	0.72
6/2/11	yes	150	HN	B3-5	T	126.82	0.00	122.70	-1.05
6/2/11	yes	150	HN	B5-2	NOT	129.96	2.14	53.76	-0.50
6/2/11	yes	150	HN	B5-2	T	119.20	2.54	57.57	0.02
6/2/11	yes	150	HN	C2-1	NOT	61.17	0.00	18.65	-0.84
6/2/11	yes	150	HN	C2-1	T	24.44	0.00	25.99	-0.99
6/2/11	yes	150	HN	E4-4	NOT	98.31	4.74	50.21	-1.07
6/2/11	yes	150	HN	E4-4	T	63.84	3.51	43.22	-0.65
6/2/11	no	50	LNS	D2-4	NOT	10.79	0.00	10.62	-0.95
6/2/11	no	50	LNS	D2-4	T	36.62	0.00	10.60	-0.78
6/2/11	no	50	LNS	E5-6	NOT	7.30	0.00	6.54	0.60

DATE	15N Enrichment	N addition (kg N ha-1 yr-1)	Plot	Field ID	NOT/T	Litter Dry Mass 5 or 12 mo. (g)	Litter Dry Mass 0 mo. (g)	Litter Mass Lost (g)	% Litter Mass Remaining
6/2/11	yes	0	C	R1-6	NOT	4.36	5.99	1.63	72.71
6/2/11	yes	0	C	R1-6	T	4.16	6.01	1.85	69.28
6/2/11	yes	0	C	R2-4	NOT	4.68	5.99	1.31	78.07
6/2/11	yes	0	C	R2-4	T	3.79	5.96	2.17	63.65
6/2/11	yes	0	C	R4-3	NOT	4.55	6.01	1.46	75.76
6/2/11	yes	0	C	R4-3	T	4.28	6.03	1.75	71.03
6/2/11	yes	50	LNS	C5-3	NOT	3.70	5.97	2.27	61.95
6/2/11	yes	50	LNS	C5-3	T	3.81	6.00	2.19	63.43
6/2/11	yes	50	LNS	D2-1	NOT	3.46	6.02	2.56	57.51
6/2/11	yes	50	LNS	D2-1	T	3.74	6.00	2.27	62.25
6/2/11	yes	50	LNS	E3-5	NOT	4.99	6.03	1.04	82.71
6/2/11	yes	50	LNS	E3-5	T	4.29	6.00	1.71	71.42
6/2/11	yes	50	LNS	E5-5	NOT	4.14	6.00	1.86	68.99
6/2/11	yes	50	LNS	E5-5	T	4.04	6.03	1.99	67.07
6/2/11	yes	150	HN	B3-5	NOT	5.07	6.02	0.95	84.26
6/2/11	yes	150	HN	B3-5	T	4.62	6.06	1.44	76.28
6/2/11	yes	150	HN	B5-2	NOT	4.70	5.97	1.27	78.75
6/2/11	yes	150	HN	B5-2	T	5.30	6.06	0.76	87.42
6/2/11	yes	150	HN	C2-1	NOT	3.63	6.02	2.39	60.24
6/2/11	yes	150	HN	C2-1	T	3.30	6.02	2.72	54.74
6/2/11	yes	150	HN	E4-4	NOT	3.25	6.02	2.77	53.95
6/2/11	yes	150	HN	E4-4	T	3.45	6.00	2.55	57.45
6/2/11	no	50	LNS	D2-4	NOT	4.39	5.97	1.58	73.46
6/2/11	no	50	LNS	D2-4	T	4.68	5.99	1.31	78.12
6/2/11	no	50	LNS	E5-6	NOT	4.54	5.99	1.45	75.80

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	^{15}N	%N	^{13}C	%C	C:N	Total N (g bag^{-1})	N gained (g bag^{-1})	Total C (g bag^{-1})
6/2/11	yes	0	C	R1-6	NOT	-2.22	2.09	-28.65	51.83	24.80	0.091	0.036	2.257
6/2/11	yes	0	C	R1-6	T	-2.46	1.35	-28.59	51.43	38.06	0.056	0.001	2.141
6/2/11	yes	0	C	R2-4	NOT	-2.25	1.54	-28.77	52.21	33.95	0.072	0.017	2.442
6/2/11	yes	0	C	R2-4	T	-2.92	1.55	-28.65	53.11	34.36	0.059	0.004	2.015
6/2/11	yes	0	C	R4-3	NOT	-2.39	1.33	-28.63	52.02	39.16	0.060	0.005	2.369
6/2/11	yes	0	C	R4-3	T	-2.64	1.71	-28.82	50.79	29.68	0.073	0.018	2.175
6/2/11	yes	50	LNS	C5-3	NOT	-2.24	1.83	-28.83	51.77	28.25	0.068	0.013	1.915
6/2/11	yes	50	LNS	C5-3	T	-2.12	1.71	-28.89	51.44	30.00	0.065	0.010	1.958
6/2/11	yes	50	LNS	D2-1	NOT	-2.00	1.75	-28.65	50.95	29.18	0.060	0.005	1.764
6/2/11	yes	50	LNS	D2-1	T	-2.32	1.77	-28.34	51.87	29.28	0.066	0.011	1.937
6/2/11	yes	50	LNS	E3-5	NOT	-2.23	1.70	-28.90	51.20	30.19	0.085	0.030	2.554
6/2/11	yes	50	LNS	E3-5	T	-2.13	1.52	-29.08	51.46	33.90	0.065	0.010	2.205
6/2/11	yes	50	LNS	E5-5	NOT	-2.19	1.51	-28.29	52.19	34.51	0.063	0.008	2.160
6/2/11	yes	50	LNS	E5-5	T	-2.57	1.58	-29.18	51.42	32.54	0.064	0.009	2.080
6/2/11	yes	150	HN	B3-5	NOT	-2.10	1.91	-29.30	51.76	27.06	0.097	0.042	2.626
6/2/11	yes	150	HN	B3-5	T	-0.76	1.78	-29.51	52.33	29.45	0.082	0.027	2.419
6/2/11	yes	150	HN	B5-2	NOT	-1.77	1.64	-29.67	51.78	31.58	0.077	0.022	2.434
6/2/11	yes	150	HN	B5-2	T	-2.13	1.48	-28.94	52.68	35.68	0.078	0.023	2.791
6/2/11	yes	150	HN	C2-1	NOT	-2.11	1.81	-29.13	52.07	28.76	0.066	0.011	1.888
6/2/11	yes	150	HN	C2-1	T	9.87	2.11	-28.92	52.44	24.91	0.069	0.014	1.728
6/2/11	yes	150	HN	E4-4	NOT	-2.11	2.25	-29.58	51.52	22.90	0.073	0.018	1.673
6/2/11	yes	150	HN	E4-4	T	-0.58	2.16	-28.03	50.91	23.57	0.074	0.019	1.755
6/2/11	no	50	LNS	D2-4	NOT	-2.09	1.46	-28.75	51.27	35.21	0.064	0.009	2.248
6/2/11	no	50	LNS	D2-4	T	-1.95	1.52	-28.62	52.88	34.77	0.071	0.016	2.474
6/2/11	no	50	LNS	E5-6	NOT	-2.10	1.42	-28.68	51.69	36.35	0.065	0.010	2.347

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	C lost (g bag^{-1})	Ergosterol (ug g^{-1} litter)	Fungal Biomass-C (mg g^{-1} litter)	Fungal Biomass-C (mg bag^{-1})	Fungal Biomass-C (% of bag)
6/2/11	yes	0	C	R1-6	NOT	0.833	360.2	32.42	141.20	3.24
6/2/11	yes	0	C	R1-6	T	0.949	272.3	24.51	102.03	2.45
6/2/11	yes	0	C	R2-4	NOT	0.648	350.2	31.52	147.39	3.15
6/2/11	yes	0	C	R2-4	T	1.075	302.8	27.25	103.38	2.72
6/2/11	yes	0	C	R4-3	NOT	0.721	196.0	17.64	80.33	1.76
6/2/11	yes	0	C	R4-3	T	0.915	357.3	32.15	137.71	3.22
6/2/11	yes	50	LNS	C5-3	NOT	1.175	235.9	21.23	78.53	2.12
6/2/11	yes	50	LNS	C5-3	T	1.132	283.1	25.48	96.98	2.55
6/2/11	yes	50	LNS	D2-1	NOT	1.326	225.9	20.33	70.40	2.03
6/2/11	yes	50	LNS	D2-1	T	1.153	298.8	26.89	100.43	2.69
6/2/11	yes	50	LNS	E3-5	NOT	0.536	346.3	31.17	155.44	3.12
6/2/11	yes	50	LNS	E3-5	T	0.885	333.7	30.03	128.69	3.00
6/2/11	yes	50	LNS	E5-5	NOT	0.930	275.0	24.75	102.44	2.47
6/2/11	yes	50	LNS	E5-5	T	1.010	363.2	32.69	132.21	3.27
6/2/11	yes	150	HN	B3-5	NOT	0.464	349.1	31.41	159.35	3.14
6/2/11	yes	150	HN	B3-5	T	0.671	363.2	32.69	151.12	3.27
6/2/11	yes	150	HN	B5-2	NOT	0.656	520.4	46.84	220.20	4.68
6/2/11	yes	150	HN	B5-2	T	0.299	352.8	31.75	168.20	3.18
6/2/11	yes	150	HN	C2-1	NOT	1.202	319.7	28.78	104.35	2.88
6/2/11	yes	150	HN	C2-1	T	1.362	217.8	19.60	64.59	1.96
6/2/11	yes	150	HN	E4-4	NOT	1.417	369.5	33.25	108.00	3.33
6/2/11	yes	150	HN	E4-4	T	1.335	202.0	18.18	62.68	1.82
6/2/11	no	50	LNS	D2-4	NOT	0.842	228.7	20.58	90.27	2.06
6/2/11	no	50	LNS	D2-4	T	0.616	323.9	29.15	136.39	2.91
6/2/11	no	50	LNS	E5-6	NOT	0.743	218.9	19.70	89.46	1.97

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Bulk Density (g cm^{-3})	Soil pH	Soil Moisture Content	Litter Moisture Content
6/2/11	no	50	LNS	E5-6	T			1.85	0.12
6/2/11	no	150	HN	B3-4	NOT			2.17	0.50
6/2/11	no	150	HN	B3-4	T			2.49	0.40
6/2/11	no	150	HN	C2-5	NOT			1.74	0.40
6/2/11	no	150	HN	C2-5	T			2.11	0.85

DATE

	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	NH4-N (KCl) ug g^{-1} soil	NO3-N (KCl) ug g^{-1} soil	NH4-N (H_2O) ug g^{-1} soil	NO3-N (H_2O) ug g^{-1} soil
6/2/11	no	50	LNS	E5-6	T	9.25	0.00	12.54	-0.65
6/2/11	no	150	HN	B3-4	NOT	22.17	1.14	28.03	-0.71
6/2/11	no	150	HN	B3-4	T	72.51	2.72	46.91	-1.39
6/2/11	no	150	HN	C2-5	NOT	11.55	0.00	16.65	1.44
6/2/11	no	150	HN	C2-5	T	7.81	0.00	23.75	-1.14

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	Litter Dry Mass 5 or 12 mo. (g)	Litter Dry Mass 0 mo. (g)	Litter Mass Lost (g)	% Litter Mass Remaining
6/2/11	no	50	LNS	E5-6	T	4.98	5.95	0.97	83.71
6/2/11	no	150	HN	B3-4	NOT	3.95	6.04	2.09	65.38
6/2/11	no	150	HN	B3-4	T	4.67	6.00	1.33	77.91
6/2/11	no	150	HN	C2-5	NOT	3.78	5.99	2.21	63.06
6/2/11	no	150	HN	C2-5	T	3.58	6.01	2.43	59.57

DATE

	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	^{15}N	%N	^{13}C	%C	C:N	Total N (g bag^{-1})	N gained (g bag^{-1})	Total C (g bag^{-1})
6/2/11	no	50	LNS	E5-6	T	-2.60	1.23	-29.41	51.69	42.01	0.061	0.006	2.574
6/2/11	no	150	HN	B3-4	NOT	-2.07	1.80	-29.00	51.59	28.69	0.071	0.016	2.037
6/2/11	no	150	HN	B3-4	T	-2.63	1.71	-29.29	51.92	30.36	0.080	0.025	2.427
6/2/11	no	150	HN	C2-5	NOT	-2.05	1.83	-28.71	50.69	27.66	0.069	0.014	1.915
6/2/11	no	150	HN	C2-5	T	-2.03	1.81	-28.21	51.19	28.23	0.065	0.010	1.832
5/18/10	no	Fresh	Leaves	1		-2.58	0.92	-29.43	51.09	56	0.05		3.07
5/18/10	no	Fresh	Leaves	2		-2.50	0.93	-29.50	52.00	56	0.06		3.12

DATE	^{15}N Enrichment	N addition (kg N ha^{-1} yr^{-1})	Plot	Field ID	NOT/T	C lost (g bag^{-1})	Ergosterol (ug g^{-1} litter)	Fungal Biomass-C (mg g^{-1} litter)	Fungal Biomass-C (mg bag^{-1})	Fungal Biomass-C (% of bag)
6/2/11	no	50	LNS	E5-6	T	0.516	239.8	21.58	107.49	2.16
6/2/11	no	150	HN	B3-4	NOT	1.053	346.1	31.15	122.99	3.11
6/2/11	no	150	HN	B3-4	T	0.663	404.7	36.42	170.26	3.64
6/2/11	no	150	HN	C2-5	NOT	1.175	211.9	19.07	72.02	1.91
6/2/11	no	150	HN	C2-5	T	1.258	212.3	19.11	68.40	1.91